Clinical and Genetic Studies in Autosomal Recessive Ataxias

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"Writing a book is an adventure. To begin with, it is a toy and an amusement.
Then it becomes a mistress, then it becomes a master, then it becomes a tyrant.
The last phase is that just as you are about to be reconciled to your servitude,
you kill the monster and fling it to the public."
-Winston S Churchill (1874-1965)

"So che si potria far presto, ma presto e bene insieme non conviene." (1 maggio 1627)
-Claudio Monteverdi (1567-1643),
letter to his patron & employer, Vicenzo Gonzaga, Duke of Mantua

Declaration

This thesis is submitted in partial fulfilment of the requirements of the regulations of the degree of Doctor of Philosophy of University College London. The author declares that, other than where stated in the text, the work is all his own. The EFACTS project was funded from the Seventh Framework Programme (FP7) of the European Commission. Further funding was received from Ataxia Ireland. The ARSACS project received funding from Ataxia UK and the *Association de l'Ataxie Charlevoix-Saguenay.*

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Abstract

167 patients with the recessive repeat expansion disorder Friedreich's ataxia (FRDA) were recruited as part of the European FRDA Consortium for Translational Studies (EFACTS) and underwent longitudinal clinical assessment including validated and standardized clinical and functional rating scales. The mean age at onset was 13.7±9.6 years (range 1-55) and disease duration 20.5±11.2 years (range 3-55). The smaller repeat expansion (GAA1 size) correlated with age at onset, Activities of Daily Living (ADL), Scale for the Assessment and Rating of Ataxia (SARA), Inventory of Non-Ataxic Symptoms (INAS) count & Spinocerebellar Degeneration Functional Score (SDFS). 125 patients were seen after 1 year, and 116 after 2 years. Disease progression could be measured using these rating scales: SARA increased over 2 years by 1.3±3.1, ADL by 2.0±3.2 and SDFS by 0.3±0.6. There was no statistical difference in INAS count. A majority of patients could not complete the Spinocerebellar Ataxia Functional Index (SCAFI) which was deemed inappropriate in FRDA. Two novel *FXN* mutations were identified, as well as a probable macrodeletion. No compound heterozygous exonic deletions were found amongst 1768 cases referred with a possible diagnosis of FRDA, indicating that these deletions are extremely rare.

Twenty-six patients with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) were recruited (mean age onset 15.0±17.4, range 0-51); mean disease duration 28.5±12.9, range 8-56). Loss of mobility, dysarthria, dysphagia, ataxia, sensory loss, square wave jerks and saccadic dysmetria were less common in ARSACS compared to FRDA; nystagmus, spasticity and hyperreflexia were more common. Nine novel *SACS* mutations were identified. Retinal Nerve Fibre Layer (RNFL) thickening on ocular coherence tomography (OCT) was found to be a specific (99.4%) and sensitive (100%) marker of ARSACS with positive predictive value of 94.4%, amongst 191 patients with ataxia, using a cut-off thickness of 119µm.

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Abbreviations

8mTW	8-metre timed walk
9HPT	9-hole peg test
$\Delta\Psi_m$	Mitochondrial membrane potential
ABN	Association of British Neurologists
ACoQ$_{10}$D	Ataxia with co-enzyme Q$_{10}$ deficiency
ADCK3	AarF domain-containing kinase 3
ADL	Activities of Daily Living
ANOVA	Analysis of variance
AOA	Ataxia with oculomotor apraxia
ARSACS	Autosomal recessive spastic ataxia of Charlevoix-Saguenay
ASMN	Axonal sensorimotor neuropathy
BL	Baseline visit
BMI	Body mass index
bp	Base pair
BP	Blood pressure
CCCP	Carbonyl cyanide *m*-chlorophenylhydrazone
CCFS	Composite cerebellar functional severity score
CI	Confidence interval
CMAP	Compound motor action potential
CMT	Charcot-Marie-Tooth
CT	Computerized tomography
DASMN	Demyelinating axonal sensorimotor neuropathy
dbSNP	Database of Single Nucleotide Polymorphisms
DNA	Deoxyribose nucleic acid
DnaJ	J-domain
DQ	Dosage quotient
DRG	Dorsal root ganglia
DSMN	Demyelinating sensorimotor neuropathy
DTI	Diffusion tensor imaging
EA	Episodic ataxia
ECG	Electrocardiogram
EDQ	Exon dosage quotient
EDTA	Ethylenediamine tetra-acetic acid
EEG	Electroencephalogram
EF	Ejection fraction
EFACTS	European Friedreich's Ataxia Consortium for Translational Studies
EQ-5D	EuroQoL 5-dimension

ESP	Exome Sequencing Project
EU	European Union
FARR	Friedreich's ataxia with retained reflexes
FARS	Friedreich's Ataxia Rating Scale
FGF	Fibroblast growth factor
FPR	False positive rate
FRDA	Friedreich's ataxia
FU1	1st follow-up visit
FU2	2nd follow-up visit
FXTAS	Fragile X tremor ataxia syndrome
GAA	Guanine-adenine-adenine
GABA	γ-aminobutyric acid
GCK	Glucokinase
GERP	Genome Evolutionary Rate Profiling
GORD	Gastro-oesophageal reflux disease
HEPN	Higher eukaryote and prokaryote nucleotide binding domain
HGMD	Human Gene Mutation Database
HSP	Heat shock protein
HSP	Hereditary spastic paraparesis
ICARS	International Cooperative Ataxia Rating Scale
INAS	Inventory of non-ataxic symptoms
IRP1	Iron regulatory protein 1
ISC	Iron-sulphur cluster
ISCED	International Standard Classification of Education
IscS	Iron-sulphur cluster cysteine desulphurase
IscU	Iron-sulphur cluster scaffold protein
IVSd	Interventricular septal thickness at diastole
JPS	Joint position sense
kbp	Kilo-base pair
LHON	Leber's hereditary optic neuropathy
LL	Lower limb
LOVD	Leiden Open Variation Database
LOFA	Late-onset Friedreich's ataxia
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVPWd	Left ventricular posterior wall thickness at diastole
MAF	Minor allele frequency
MANCOVA	Multivariate analysis of covariance
MARS	Methionyl-tRNA transferase

MCP	Middle cerebellar peduncle
MCV	Motor conduction velocity
MLPA	Multiplex ligation-dependent probe amplification
MoCA	Montreal Cognitive Assessment
MODY2	Maturity-onset diabetes of the young type 2
MPP	Mitochondrial processing peptidase
mRNA	Messenger ribose nucleic acid
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MSA	Multiple sequence alignment
MSA-C	Multisystem atrophy, cerebellar type
mtDNA	Mitochondrial deoxyribose nucleic acid
NAION	Non-arteritic anterior ischaemic optic neuropathy
NCBI	National Center for Biotechnology Information
NCS	Nerve conduction studies
NFH	Heavy-chain neurofilament
NGRL	National Genetics Reference Laboratory
NHLBI	National Heart, Lung and Blood Institute
NHNN	National Hospital for Neurology and Neurosurgery
nt	Nucleotide
OCT	Ocular coherence tomography
PCR	Polymerase chain reaction
PGM5	Phosphoglucomutase-5
PhastCons	Phylogenetic Analysis with Space/Time models Conservation Program
PhyloP	Phylogenetic p-values
PIC	Participant identification centre
PIP5K1B	Phosphatidylinositol-4-phosphate-5-kinase type 1β
PIS	Participant information sheet
PolyPhen2	Polymorphisms Phenotyping version 2
PRKACG	Protein kinase cAMP-dependent catalytic γ
RAPD	Relative afferent pupillary defect
RCPCH	Royal College of Paediatrics and Child Health
RNFL	Retinal nerve fibre layer
SALSA	Selective Adaptor Ligation, Selective Amplification
SAP	Sensory action potential
SARA	Scale for the Assessment and Rating of Ataxia
SCA	Spinocerebellar ataxia
SCAFI	Spinocerebellar Ataxia Functional Index
SCV	Sensory conduction velocity

SD	Standard deviation
SD-OCT	Spectral domain ocular coherence tomography
SDFS	Spinocerebellar Degeneration Functional Score
SIFT	Sorting Intolerant From Tolerant
siRNA	Small interfering ribose nucleic acid
SLS	Sögren-Larsson syndrome
SMRNF	Syndrome of myelinated retinal nerve fibres
SNE	Structured neurological examination
SNP	Single nucleotide polymorphism
SPECT	Single photon emission computed tomography
SPG	Spastic paraparesis gene
SSR	Sacsin repeat region
SWJ	Square wave jerk
TAT	Transactivator of transcription
TD-OCT	Time domain ocular coherence tomography
TE	Tris-EDTA buffer
TMEM252	Transmembrane protein 252
TMRM	Tetramethylrhodamine
TRPV4	Transient receptor potential cation channel subfamily V member 4
UBL	Ubiquitin-like domain
UK	United Kingdom
UL	Upper limb
UNESCO	United Nations Educational, Scientific and Cultural Organization
VAS	Visual analogue scale
VLOFA	Very late-onset Friedreich's ataxia
WHO	World Health Organization
XPCB	Xeroderma pigmentosum complementation group C binding domain

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Chapter 1 : Friedreich's Ataxia

1.1 Introduction

2013 marked the 150th anniversary of the seminal description by Nikolaus Friedreich of the ataxic syndrome which now bears his name (Friedreich 1863c, Friedreich 1863a, Friedreich 1863b). Since then, descriptions of its clinical phenotype have evolved and extended, but it was not until the 1970s and 1980s that systematic phenotypic descriptions allowed the development of reliable diagnostic clinical criteria (Geoffroy *et al.* 1976, Harding 1981) (see Table 1). However, these cases were not genetically proven. The discovery in 1996 of the genetic abnormality responsible for FRDA (Campuzano *et al.* 1996) allowed genotype-phenotype correlations, and expanded further the previously understood phenotypic spectrum. Since then, there have been few natural history studies performed on large, genetically confirmed series of patients with FRDA, which are so vital in illuminating underlying molecular mechanisms, in planning and directing interventional trials and in providing prognostic information for patients (see Table 2).

The European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) was formed in 2010 with the intention of assembling a body of expertise in clinical neurology, biochemistry, molecular biology, genetics and bioinformatics which could apply a translational approach to the study of FRDA. Central to this objective was the recruitment of a large pan-European cohort of patients with genetically proven FRDA whose presentation and progression would be systematically and prospectively recorded, and who would form part of a patient registry linked to a repository of biological samples collected during the study which would enable patients' participation in therapeutic trials and studies of fundamental pathological mechanisms. The UK contribution to this project forms the core of this thesis. This introductory chapter reviews the history and chronology of our developing understanding of this condition, describes current concepts of pathogenic mechanisms and summarizes current knowledge of its clinical features.

Table 1: Diagnostic criteria for FRDA

	Geoffroy, *et al.* (1976)	Harding (1981)
Primary criteria (essential for diagnosis)	• Onset before end of puberty and never after age 20 • Ataxia of gait • Progression of ataxia within last 2 years to all extremities without remission • Dysarthria • Decreased position and/or vibration sense in lower limbs • Muscle weakness • Lower limb deep tendon areflexia	• Onset before age 25 • Progressive unremitting ataxia of limbs and gait • Absent knee and ankle jerks
Secondary criteria	• Extensor plantar responses • Pes cavus • Scoliosis • Cardiomyopathy	• Dysarthria • Extensor plantar responses
Additional criteria	• Decrease in visual acuity • Nystagmus • Paresthesiae • Partial deafness • Essential type tremor • Vertigo • Spasticity • Pain • Decrease in IQ	If secondary features not present, then must have : • An affected sibling with both secondary criteria ; or • Median motor nerve conduction velocities > 40m/s

Table 2: Frequencies of clinical features in published cases series of FRDA

	Geoffroy *et al.* 1976; Andermann *et al* 1976	**Harding 1981**	**Ackroyd *et al.* 1984**	**Filla *et al.* 1990**	**Dürr *et al.* 1996**	**Schöls *et al.* 1997**	**Lamont *et al.* 1997**	**Delatycki *et al.* 1999**	**McCabe *et al.* 2000**
Origin	Canada	UK	UK	Italy	France & Réunion	Germany	UK	Australia	Ireland
Genetic diagnosis	No	No	No	No	Yes	Yes	Yes	Yes	Yes
No. of patients	36[b] ; 58	115	12	80	140	38	56	51	58
Male:female	1 : 2.0	1 : 1.2	1 : 1.0	1 : 0.7	1 : 1.1	1 : 1.4	-	1 : 0.8	-
Age at onset (years)[a]	7.97±3.07	10.52±7.4 (1.7-27)	5.8 (3-9)	11.6±4.5 (2-23)	15.5±8 (2-51)	14.15±5.03 (5-36)	(3-30)	10.5±6.4 (1-26)	-
Disease duration (years)[a]	11.72±7.04	22.00±12.76 (2-61)	4.7 (1-10)	13.4	15.5	19.69±8.75 (5-42)	13.6±9.9 (6-50)	-	-
Age when wheelchair-bound (years)[a]	-	25.14±15.5 (11-58)	-	26.3±7.8 (15-42)	26.3	23.98±5.66 (15-44)	-	19.0±6.4 (8-33)	-
Age at onset to age at wheelchair-bound (years)[a]	-	15.51±7.41 (3-44)	-	13.8±5.8	10.8±6 (1-25)	11.29±4.09 (5-26)	-	10.1±4.4 (1-20)	-
Wheelchair-bound (%)	-	72	-	43	-	78	-	55	-
Age at death (years)[a]	30.6±1.3 (4.5-40)	37.54±14.35 (21-69)	-	13.5±3.5 (10-17)	-	-	-	-	-
Gait ataxia (%)[c]	100	100	100	100	100	100	-	100	100
Limb ataxia (%)	-	99	100	94	99	100	100	100	-
Dysarthria (%)	100	97	75	84	91	100	91	95	93
Dysphagia (%)	-	-	-	30	27	76	-	-	-

Nystagmus (%)	42	20	25	29	40	39	-	-	40
Broken pursuit eye movements (%)	-	12	-	-	30	-	-	-	52
LL areflexia (%)	100	99	100	100	87	84	87	98	86
Extensor plantar responses (%)	94	89	42	75	79	95	96	74	93
Loss of vibration sense (%)	-	73	67	91	78	83	87	88	89
LL muscle weakness (%)	100	88	92	80	67	69	-	-	72
UL amyotrophy (%)	-	49	42	49	25	-	-	-	29
LL amyotrophy (%)	-	39	92	61	39	50	-	-	67
Scoliosis (%)	92	79	33	94	60	84	-	78	84
Foot deformity (%)[d]	89	55	83	90	55	82	-	74	79
Cardiomyopathy (%)[e]	97	-	100	28	63	75	-	65	67
Diabetes (%)[f]	19	10	-	14	32	6	-	8	7
Sphincter disturbance (%)	7	-	-	18	23	12	-	41	-
Hearing loss (%)	22	8	-	9	13	39	-	-	
Visual loss (%)	50	18	-	-	13	6	-	-	-

[a] Expressed as mean±SD (range)
[b] 'Typical' FRDA with complete or incomplete picture
[c] Gait ataxia present or not testable due to weakness
[d] Pes cavus, talipes equinovarus or other foot abnormality
[e] Cardiomyopathy or left ventricular hypertrophy (definitions vary between papers) [f] Diabetes or impaired glucose tolerance (definitions vary between papers)
- Not known or not recorded
UL=upper limb ; LL=lower limb

1.2 History

Figure 1: Nikolaus Friedreich (1825-1882)
(Universitätsbibliothek Heidelberg, http://heidicon.ub.uni-heidelberg.de/id/33455)

Nikolaus Friedreich was born in Würzburg on 31 July 1825, the son of Johannes Baptist Friedreich (1796-1862), Professor of Surgery in the Medical Faculty of the University of Würzburg, and grandson of Nikolaus Anton Friedreich (1761-1836), also Professor of Surgery at Würzburg, who had described idiopathic facial nerve paralysis in 1798 some 23 years before Sir Charles Bell in a paper named *De paralysis musculorum faciei rheumatica* (Bird 1979). Nikolaus Friedreich (1825-1882; see Figure 1) began his medical studies in Würzburg in 1844 and graduated in 1850 completing a thesis on tumours within the skull *(Beiträge zur Lehre von den Geschwülsten innerhalb der Schedelhöhle)*. He was strongly influenced by Rudolf Virchow (1821-1902) who worked in Würzburg from 1849 and whom Friedreich succeeded as Professor of Pathological Anatomy when Virchow returned to Berlin in 1856. Two years later, Friedreich was appointed chief of the medical clinic in Heidelberg where he took up the Chair of

Pathology and Therapy. His main interest was diseases of the nervous system. He remained in Heidelberg for the rest of his career, teaching such luminaries as Adolf Kussmaul (1822-1902) and Wilhelm Erb (1840-1921), the latter succeeding him as Professor of Pathology and Therapy in 1882 (Koehler 2000).

The term ataxia meaning disorder, irregularity or confusion (ατάξις – lack of order) had been used since the time of Hippocrates in the description of disease as well as more generally but it was not until the nineteenth century that its present meaning of incoordination of neurological origin was adopted. Guillaume Duchenne de Boulogne (1806-1875) used the term *ataxie locomotrice progressive* to describe a distinct condition characterized by incoordination of movement. At the time, a wide variety of conditions causing functional impairment attracted the epithets 'palsy', 'paralysis' or 'paraplegia' such as the 'shaking palsy' now known as Parkinson's disease. Duchenne drew the distinction between true weakness found on testing individual muscle groups and the apparent weakness resulting from incoordination of movement *('Abolition progressive de la coordination des mouvements et paralysie apparente, contrastant avec l'intégralité de la force musculaire')* (Duchenne 1858).

Ataxic disorders remain to the present day defined by their principal symptom, but the term progressive locomotor ataxia was insufficiently linked to a single pathological entity and hence this appellation and the term Duchenne's disease are no longer used. Indeed, by 1895, William Gowers commented in his Clinical Lectures at the National Hospital for the Paralysed and Epileptic, that the term was no longer considered accurate as, 'The disease in a large number of cases is not progressive' and 'the term *"locomotor ataxy"* is itself somewhat redundant, because the disorder is one of movement in general, and not merely that which causes change of place.' (Gowers 1895). Cases of the locomotor ataxia described by Duchenne and extensively in the medical literature of the nineteenth century, were probably predominantly caused by tabes dorsalis (syphilitic myeloradiculopathy). It was not until the 1880s that the epidemiological link between syphilis and tabes was firmly established through the works of Jean-Alfred Fournier (1832-1914), Gowers and Erb, having previously been thought to be caused by a number of environmental and hereditary factors including excessive drinking and sexual activity. Fritz Schaudinn (1871-1906) and Erich Hoffman

(1868-1959) finally discovered the underlying infective agent of syphilis in 1905, describing it as *Spirochaeta pallida*, later known as *Treponema pallidum* (Goetz 1987, Nitrini 2000, Waugh 2005).

Friedreich began his studies of ataxia in the 1850s. His critical observation was to realize that a subgroup of his patients were not suffering from Duchenne's progressive locomotor ataxia, but from a hereditary form associated with early age at onset, long duration and absence of sensory loss in the early stages. He also felt there was a female predominance, although this was not borne out by subsequent studies. Other typical symptoms of tabes such as pupillary disorders (Argyll-Robertson pupils), shooting lancinating pains, joint disorders and onset in later life, were not present. He presented his initial findings at the meeting of the Society of German Natural Scientists and Physicians *(Versammlung Deutscher Naturforscher und Ärzte)* in Speyer close to Heidelberg, on the 18th September 1861. He subsequently published the cases of six named patients (Andreas and Charlotte Lotsch; Justine, Salome, Lisette and Friedrich Süss) from two families including three autopsies in a series of monographs in 1863 (Friedreich 1863c, Friedreich 1863a, Friedreich 1863b):

> The clinical characters of the disease are impairment in the coordination and harmony of movements, developing gradually and spreading from the lower to the upper half of the body, and always involving finally the organs of speech; sensibility and the functions of the special senses, and of the brain, being intact…Less common phenomena are curvature of the spine, sensations of vertigo, and nystagmus. From a clinical point of view we must regard the disease as a progressive paralysis of the faculty of the combination of movements; from the point of view of pathological anatomy, as a chronic degenerative atrophy of the posterior columns of the cord.
>
> *Die Affection ist klinisch ausgezeichnet durch eine, in sehr allmäligem Verlaufe sich entwickelnde, von der unteren auf die obere Körperhälfte forterstreckende, constant zuletzt auch die Sprachorgane betheiligende Störung in der Association und Harmonie der Bewegungen, bei ungestörter Sensibilität und bei vollständiger lntegrität der Sinnesorgane und cerebralen Functionen… Als weniger constante Erscheinungen sind Verkrümmungen der Wirbelsäule, Schwindelgefühle und Nystagmus zu nennen. Die Krankheit dürfte vom klinischen Gesichtspunkte aus als chronische progressive Lähmung der Combination der Bewegungen, vom pathologisch-anatomischen Standpunkte aus als chronische degenerative Atrophie der spinalen Hinterstränge zu bezeichnen sein* (Friedreich 1863a).

His proposition that he had described a discreet nosological entity was disputed for many years. Most authors, such as Wilhelm Erb (1840-1921), Adolph Strümpell (1853-1925), Paul Topinard (1830-1911), Albert Eulenburg (1840-1917), Paul Julius Möbius (1853-1907) and Sigismond Jaccoud (1830-1913), felt that it represented a hereditary or juvenile form of tabes dorsalis. Jean-Martin Charcot (1825-1893) and Désiré-Magloire Bourneville (1840-1909) felt it was caused by multiple sclerosis *(la sclerose en plaques disséminées)* which Charcot and Alfred Vulpian (1826-1887) had described in 1868 (Keppel Hesselink 1986). Bourneville specifically re-examined two of Friedreich's cases commenting about that of Justine Süss that:

> A careful reading of this observation easily proves that, from the clinical point of view, the picture is far from being completely that of progressive locomotor ataxia. The nature of the onset described is not that normally seen in ataxia, while it is much more in line with that of multiple sclerosis. The impairment of speech, the tremor of the head, the nystagmus, are morbid phenomena foreign to the classical type of ataxia. Finally, contrary to what takes place so frequently in this disease, cutaneous sensibility and vision, were not affected.
>
> *La lecture attentive de cette observation prouve sans peine que, au point de vue clinique, le tableau est loin d'être complètement celui de l'ataxie locomotrice progressive. Le mode de début indiqué n'est pas celui qui se voit d'ordinaire dans l'ataxie, tandis qu'il est beaucoup plus conforme à celui de la sclérose en plaques. L'embarras de la parole, le tremblement de la tête, le nystagmus, sont des phénomènes morbides étrangers au type classique de l'ataxie. Enfin, contrairement à ce qui a lieu si fréquemment dans cette maladie, la sensibilité cutanée, la vision n'étaient point affectées* (Bourneville & Guérard 1869).

and of his sister Salome Süss that:

> The value of this result will not escape anyone. The reality of the sclerotic lesions, even though they escape the naked eye, confirming what we said in a previous chapter, has given a considerable contribution to the opinion we stated, namely the coexistence in this case, of progressive locomotor ataxia and multiple sclerosis.
>
> *La valeur de ce résultat n'échappera à personne. La réalité des lésions scléreuses, alors qu'elles échappent à l'oeil nu, en confirmant ce que nous avons dit dans un chapitre précédent, vient donner un appoint considérable à l'opinion que nous avons émise, à savoir, la coexistence, dans ce cas, de l'ataxie locomotrice progressive et de la sclérose en plaques disséminées* (Bourneville & Guérard 1869).

In 1876 and 1877 Friedreich published the cases of three more sisters (Louise, Katharina and Marie Schulz) and further information on those previously described (Friedreich 1876, Friedreich 1877). In particular, he countered Bourneville's and Charcot's assertion that his cases represented mixed forms with multiples sclerosis and that Bourneville had not discussed his most characteristic case:

> Bourneville in fact says that the cases described by me were not pure ataxias but mixed forms with multiple sclerosis, and Charcot agrees with this opinion… Bourneville has reproduced in detail in his work, cases III and IV described by me (Justine and Salome Süss), while my case I (Andreas Lotsch), although it showed most clearly the clinical and anatomical features of posterior column degeneration, strangely remained unrecorded. Charcot thinks that the speech disturbance and the nystagmus observed in my cases are mainly symptoms characteristic of multiple sclerosis and, on the other hand, are not seen or only in exceptional cases in degeneration of the posterior columns, the evidence provided that the cases were not pure ataxias. I have no hesitation in recognizing the rarity of both symptoms mentioned in the common forms of ataxic tabes; but I must explain that I am firmly against the idea that this cannot also occur in pure and uncomplicated degeneration of the posterior columns, and in particular the hereditary forms of ataxia described by me are characterized by the existence of those symptoms in the common forms, as I emphasized repeatedly.
>
> *Bourneville spricht sich nehmlich dahin aus, dass die von mir geschilderten Fälle keine reinen Ataxien, sondern Mischformen mit multipler Sclerose gewesen seien, und auch Charcot schliesst sich dieser Meinung an…. Bourneville hat in seiner Arbeit die von mir geschilderten Fälle III und IV (Justine und Salome Süss) ausführlich wiedergegeben, während mein Fall 1 (Andreas Lotsch), obgleich gerade bei diesem die klinischen und anatomischen Verhältnisse der Hinterstrangdegeneration am Reinsten hervortraten, sonderbarer Weise unberücksichtigt geblieben ist. Charcot meint, dass die in meinen Fällen beobachtete Störung der Sprachbewegungen und der Nystagmus, welche Symptome vorwiegend der multiloculären Sclerose eigenthümlich seien, dagegen nicht oder nur ausnahmsweise der Degeneration der Hinterstränge zukämen, den Beweis lieferten, dass dieselben nicht reine Ataxien gewesen seien. Ich nehme keinen Anstand, die Seltenheit der genannten beiden Symptome für die gewöhnlichen Formen atactischer Tabes anzuerkennen; allein ich muss mich eben so entschieden dagegen erklären, dass dieselben nicht auch bei reiner und uncomplicirter Degeneration der Hinterstränge vorkommen können, und gerade die von mir geschilderten Formen hereditärer Ataxie sind, wie ich dies wiederholt betonte, durch das Vorhandensein jener Symptome von den gewöhnlichen Formen ausgezeichnet* (Friedreich 1876).

He concluded:

> I find it incomprehensible that anyone can still speak of multiple sclerosis, when I have given the results of 3 carefully made autopsies… I have not given up hope that Charcot in the vast record of observations at his disposal, would sooner or later have the opportunity of finding cases analogous to those described by me.
>
> *(I)ch finde es für unbegreiflich, wie man Angesichts der von mir mitgetheilten 3 Sectionsergebnisse von multipler Sclerose sprechen kann… (I)ch gebe die Hoffnung nicht auf, dass Charcot bei dem ihm zu Gebote stehenden grossen Beobachtungsmateriale früher oder später einmal analoge Fälle, wie die von mir beschriebenen, zu sehen Gelegenheit finden werde* (Friedreich 1876).

It was not until 1882 however that the French neurologist Auguste Brousse working in Montpellier definitively acknowledged Friedreich's hereditary ataxia as a distinct nosological entity for which he proposed the term *'la maladie de Friedreich.'* (Brousse 1882):

> The disease studied by Friedreich under the name hereditary ataxia, is a specific disease with an aetiology, symptomatology and pathological anatomy of its own ... As for the term hereditary ataxia, it seems quite inappropriate, given the nature of the disease. We would prefer FRIEDREICH'S DISEASE, as we should give the name Duchenne's disease to classic progressive locomotor ataxia.
>
> *La maladie étudiée par Friedreich sous le nom d'ataxie héréditaire est une maladie spéciale ayant une étiologie, une symptomatologie et une anatomie pathologique propres…Quant au terme d'ataxie héréditaire, il nous paraît assez impropre, étant donné la nature spéciale de la maladie. Nous préférerions celui de MALADIE DE FRIEDREICH, de même qu'on devrait donner le nom de Maladie de Duchenne à l'ataxie locomotrice progressive classique* (Brousse 1882).

He summarized the 31 cases in the literature he felt represented cases of Friedreich's disease and added a further case with autopsy findings, a 32-year old domestic maid named Marie R employed by a baker to deliver bread. Onset was eight years before, when she noticed her legs became progressively weaker and less coordinated and her gait more hesitant. After four years, she could not walk.

Brousse defined the disease's origin as being in childhood or adolescence under direct or indirect genetic influence with the sexes affected equally. Symptomatically, it was characterized by ataxia of all four limbs, beginning in the lower limbs and progressing until it caused almost complete functional loss of power. Speech was affected but

sensory loss occurred only later. The progression was slow and long but was inevitably fatal with death occurring because of intercurrent illness:

> *Au point de vue symptomatique, par l'ataxie des quatre membres, débutant par les membres inférieurs et se généralisant ensuite au point de réduire les malades à une impotence fonctionnelle à peu près absolue, par l'embarras delà parole, par l'absence ou l'apparition tardive des troubles de sensibilité ,par l'absence de troubles trophiques et la conservation de la tonicité des sphincters, par une marche lente et constamment progressive, par une durée très longue, enfin par une terminaison toujours fatale se produisant le plus souvent par une maladie intercurrente* (Brousse 1882)

Pathologically, it was characterized by degeneration of the posterior columns up to the bulbar region with more diffuse degeneration in the lateral and anterior columns. He underlined the importance of distinguishing it from progressive locomotor ataxia, multiple sclerosis and other forms of ataxia *('elle doit être distinguée de l'ataxie locomotrice progressive, de la sclérose en plaques disséminées'*).

The term Friedreich's disease quickly caught on with Charles Féré later that year using the term '*la maladie de Friedreich'* to describe the by that time 48 cases appearing in the literature consistent with Friedreich's original description (Féré 1882). Over the next few years, the term appeared in the German, British, French, Italian, American and Russian literature (Bury 1886). Significantly, Charcot presented a young patient at the Salpêtrière in 1884 with a hereditary ataxia which he realized was not caused by multiple sclerosis or tabes dorsalis (Goetz 1987, Keppel Hesselink 1986). He subsequently presented several cases at length (Charcot 1888).

Freidreich died on 6 July 1882 at the age of 57 of a ruptured thoracic aortic aneurysm. The British Medical Journal published an editorial the following March, commenting:

> It is just twenty years since Professor Friedreich, whose recent death has deprived the University of Heidelberg of one of its greatest ornaments, first drew attention to a peculiar form of degenerative atrophy of the posterior columns of the spinal cord, to which he and others after him have applied the name "hereditary ataxy" (Anon 1883).

They, like others, favoured the term Friedreich's disease over hereditary ataxia as the cases were typically familial rather than hereditary. This concept must have been difficult to disentangle before there was a full understanding of genetics or the

infective aetiology of tabes dorsalis given that syphilis can be transmitted vertically whereas Friedreich's ataxia rarely appears in consecutive generations.

Further case series appeared with Dr Everett Smith of Framingham, Massechusetts identifying 57 cases including six of his own in 1885 (Smith 1885). Gowers described 65 cases from 19 families in his famous Manual of Diseases of the Nervous System in 1886 (Gowers 1886). Kinnier Wilson in his textbook of neurology describes 73 cases of Friedreich's disease from 15,923 admissions to the National Hospital between 1909 and 1925 (Wilson 1940). Although Friedreich described thickening of the left ventricular wall and fatty degeneration of the heart tissue in his original cases, and sporadic reports of cardiac involvement and ECG changes in Friedreich's ataxia appeared in the first half of the twentieth century, Loiseau is accredited with bringing these reports together systematically in 1938 (Loiseau 1938). The occurrence of diabetes was clearly linked with FRDA for the first time by Thorén in 1962 in his description of a cohort of 50 patients (Thorén 1962). The presence of neurological and non-neurological manifestations in FRDA made this condition a truly multisystem disorder. The causative gene was mapped to chromosome 9p22 in 1988 (Chamberlain *et al.* 1988) and fully elucidated in 1996 (Campuzano *et al.* 1996).

1.3 Genetics & Pathology

FRDA is now recognized as the commonest hereditary form of ataxia, caused by an autosomally recessively inherited unstable GAA expansion situated in intron 1 of the *FXN* gene on the proximal long arm of chromosome 9. This causes decreased production of frataxin (see Figure 2), probably through transcriptional silencing because of heterochromatin formation. Frataxin is a mitochondrial protein essential for life but whose function is not fully understood. It is thought to be important in the biogenesis of iron-sulphur clusters which, amongst other roles, act as cofactors in the mitochondrial respiratory chain. Iron-sulphur-cluster-containing enzymes include aconitase and mitochondrial complexes I to III (Martelli *et al.* 2012, Lill 2009). A small minority of cases (about 4%) carry a compound heterozygous GAA expansion with a point mutation or large deletion in the *FXN* gene (Campuzano et al. 1996, Cossée *et al.* 1999, Gellera *et al.* 2007). No homozygous point mutations or deletions have hitherto

been described. The molecular biology of frataxin and the clinical and genetic nature of compound heterozygotes with point mutations or large deletions are discussed more extensively in Chapter 3.

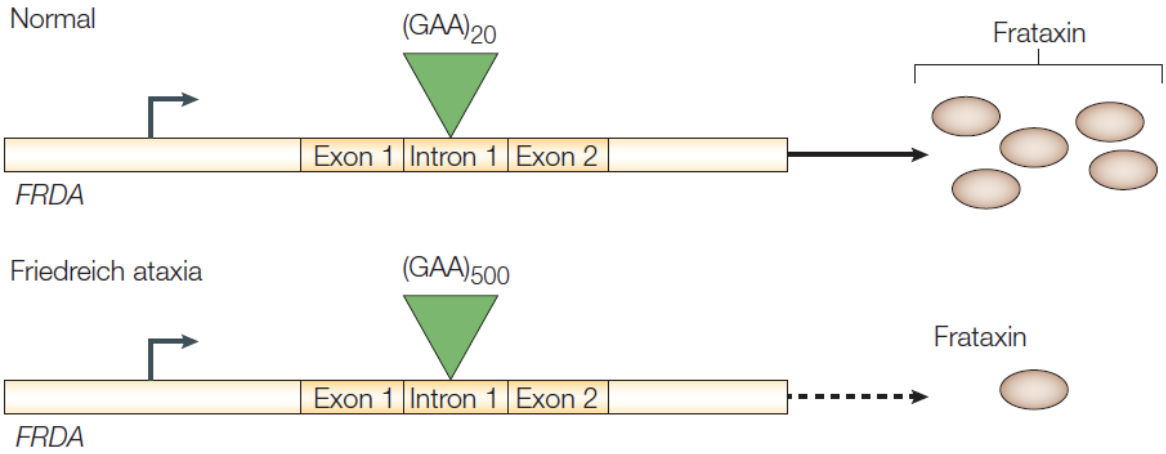

Figure 2: Schematic representation of GAA expansion in *FXN* intron 1 causing decreased frataxin production.
From (Gatchel & Zoghbi 2005)

The prevalence of the condition has been estimated in a variety of Western European populations at between 1:20,000 and 1:125,000 (Filla *et al.* 1992). Carrier frequency is estimated at between 1:60 and 1:110 (Epplen *et al.* 1997, Cossée *et al.* 1997, Harding & Zilkha 1981). The GAA expansion appears only to exist in individuals of European, North African, Middle-Eastern or Indian origin and is not described in those of Sub-Saharan African, Amerindian or East Asian origin (Labuda *et al.* 2000). Male to female distribution is equal. Families usually consist of an isolated individual or multiple siblings, although pseudo-dominance has been described (Harding & Zilkha 1981). Heterozygous carriers of the GAA expansion without any other abnormality of the *FXN* gene are not thought to be clinically affected (Harding 1981, Andermann *et al.* 1976) although they are known to produce significantly less frataxin than normal controls (Willis *et al.* 2008, Deutsch *et al.* 2010, Saccà *et al.* 2011).

The clinical phenotype of FRDA is thought to be largely determined by the effect of the GAA repeat expansion on gene transcription. The expansion size has been shown to be inversely correlated with age at onset and confinement to wheelchair, and positively correlated with the incidence of cardiomyopathy (Dürr *et al.* 1996, Filla *et al.* 1996, Montermini *et al.* 1997). The closest genotype-phenotype relationship is with the

smaller of the two alleles (GAA1), probably because large expansions produce very little frataxin, and so the smaller expansion produces the majority of the frataxin. The size of the smaller expansion therefore has a much greater influence over the total amount of frataxin produced and therefore over clinical severity. GAA1 size has been correlated with both frataxin mRNA (Pianese *et al.* 2004) and protein levels (Campuzano *et al.* 1997), with expansions of approximately 800 to 1000 GAA repeats producing approximately 10% of the normal amount of frataxin mRNA, whereas expansions of approximately 400 repeats produce approximately 40% of normal (Pianese et al. 2004). Exceptionally small or large GAA repeat sizes and compound heterozygotes with point mutations are responsible for most atypical presentations.

The smallest symptomatic non-interrupted GAA1 expansion so far described involved 44 repeats, whereas normal chromosomes have fewer than 33 repeats (Pandolfo 2001). Patients with FRDA usually have between 70 and 1500 repeats, most commonly 600 to 900 (Pandolfo 2001). Late-onset atypical FRDA phenotypes commonly have between 100 and 500 repeats in GAA1, comparable to the smaller of the two alleles in classical FRDA (Schöls *et al.* 1997, Dürr et al. 1996, Bhidayasiri *et al.* 2005). Compound heterozygotes with a missense mutation on one allele located near the amino end of the carboxy-terminal domain of frataxin may also result in an atypically mild FRDA phenotype (Cossée et al. 1999, Bhidayasiri et al. 2005).

Marked variability in symptoms between individuals and within families is seen in FRDA related to intergenerational instability of the GAA expansion (Monros *et al.* 1997). It is estimated than GAA expansion size accounts for approximately 50% of the variability in age at onset (Filla et al. 1996). Other contributory factors may include somatic mosaicism, interruptions in the repeat sequence, changes in expansion size over life and other modifying genes or environmental factors (Pandolfo & Pastore 2009). Of note, almost all human studies measure white cell GAA expansion size which is a tissue not affected clinically in FRDA.

Friedreich published detailed pathological descriptions of the spinal cord, dorsal spinal roots and medulla in his paper of 1877 (Friedreich 1877). Although he recognized that the dorsal root ganglia nerve fibres were abnormally thin, he placed more emphasis on

the degeneration of the spinal cord as well as the medulla oblongata and hypoglossal nuclei (Koeppen & Mazurkiewicz 2013) (see Figure 3). Modern analysis shows that macroscopically, the dorsal columns and dorsal root ganglia (DRG) are thin and grey, with reduced calibre of the spinal cord at all levels, particularly in the thoracic region where the transverse diameter is often less than 10mm (Koeppen 2011). There is loss of myelin and axons in the dorsal columns, as well as the spinocerebellar and corticospinal tracts. In the DRG, ganglion cell size is reduced and very large neurones are absent leaving behind residual nodules of Nageotte comprising degenerating neurones surrounded by an abnormally thickened rim of satellite cells. These show increased immunoreactivity for the iron storage and transporter proteins ferritin and ferroportin, supporting the idea that cellular iron excess may be significant in the pathology of FRDA. Axonal density is unchanged in the DRG but there is a decrease in large myelinated fibres (Koeppen *et al.* 2009). Peripheral sensory nerves show loss of myelinated fibres and a shift to thinner axons (Morral *et al.* 2010). In the cerebellum, atrophy of the dentate nucleus and its efferent fibres is seen with relatively preserved cerebellar cortex and white matter. Microscopically, there is grumose degeneration and loss of large neurones, particularly with γ-aminobutyric acid (GABA)-containing terminals implying impaired corticonuclear connections (Koeppen 2011).

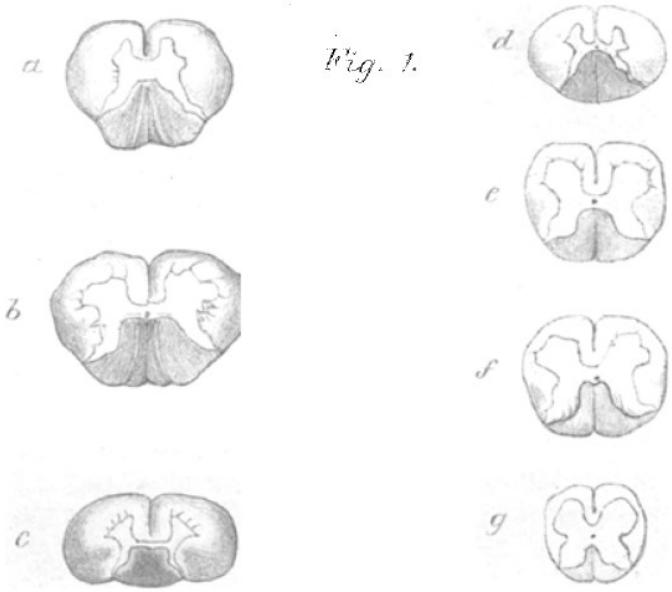

Figure 3: Serial cross sections of the spinal cord from rostral (a) to caudal (g) with the dorsal columns shown inferiorly.
Friedreich commented that *'Das ganze Rückenmark ist dünner und schmächtiger'* : the whole spinal cord is thin and slight (Friedreich 1877)

1.4 Clinical Features

1.4.1 Neurological Features

Typical age at symptom onset is around or before puberty with large case series showing mean age at onset of 10.5±7.4 years (Harding 1981), 11.6±4.5 years (Filla *et al.* 1990) and 15.5±8 years (Dürr et al. 1996). Harding showed modal age at onset of 10-12 years and Filla *et al.* (1990) 12-15 years. Age at symptom onset is always studied retrospectively and as such may be subject to significant variability in recall. Earlier non-genetic studies may also be skewed to earlier age at onset because of the use of clinical criteria requiring early age at onset. Gait ataxia and general clumsiness are the commonest presenting symptoms (Harding 1981, Filla et al. 1990, Dürr et al. 1996, Delatycki *et al.* 1999). A small proportion of patients present via orthopaedic clinics with scoliosis in which additional neurological signs may have been observed.

Gait and limb ataxia, dysarthria and lower limb (LL) areflexia are found in almost all cases, although early cases series may have overestimated their frequency because they were required clinical features in the pre-genetic diagnostic criteria (see Table 2). The ataxia is of mixed origin, resulting from spinocerebellar degeneration, peripheral sensory neuropathy, cerebellar and vestibular pathology (Delatycki & Corben 2012). Pyramidal involvement later adds to the disability.

Gait becomes unsteady and ataxic with increasing falls. Walking on uneven terrain or in poor light becomes problematical. Difficulty with tandem standing and walking is an early sign. Many patients in retrospect describe a long history of clumsiness, trips or inability to participate in sports. There is increasing dependence on aids to walking, initially furniture, walls and other people, and ultimately sticks, crutches and wheeled walkers. Harding (1981) found the mean age from symptom onset to dependence on wheelchair was 15.5 years (range 3-44). Truncal ataxia results in swaying on sitting and may necessitate back support. Romberg's test becomes positive or there may be difficulty early in disease progression with standing in tandem. Limb ataxia causes increasing difficulty with daily activities which require fine manual dexterity, and is an early feature of the disease. This causes difficulty with handwriting, washing, dressing,

use of cutlery and carrying drinks or food. Progression of weakness may mask ataxia as a clinical sign.

Pyramidal weakness is a relatively late sign and is much more prominent in the lower limbs compared to the upper limbs (ULs) : indeed, patients often have very well preserved UL power even when wheelchair-bound and profoundly disabled, and may only ever develop mild distal UL weakness. However, this can contribute significantly to difficulties with fine manual dexterity. Progression of weakness also may mask ataxia as a clinical sign. Wasting is noted in a significant proportion of cases although in patients who develop the disease in early life, muscle bulk may never be fully developed without significant loss thereafter (Harding 1981, Filla et al. 1990, Dürr et al. 1996).

Areflexia, particularly of the LLs, is an early sign present in almost all patients and reflects the underlying peripheral neuropathy. Extensor plantar reactions, reflecting pyramidal pathology, are a relatively early sign present in 73-89% of cases (Harding 1981, Dürr et al. 1996, Delatycki et al. 1999). Muscle tone is typically normal or reduced, particularly in the early stages of the disease. Spasticity, particularly in the lower limbs, can become a significant management problem in the advanced stages of the disease, especially in wheelchair-bound patients. It can cause pain, discomfort, positioning problems and ultimately contractures if left untreated. It can be associated with muscle cramps and spasms, which can often keep patients awake at night.

Distal sensory loss is virtually universal, with the dorsal column modalities of vibration and joint position sense preferentially lost, contributing to the sensory ataxia (Harding 1981, Dürr et al. 1996, Schöls et al. 1997, Delatycki et al. 1999). Neurophysiological studies show a severe axonal neuropathy with severely reduced or absent sensory action potentials (SAPs) which do not appear to change significantly over time. Peripheral nerve biopsy shows an increase in proportion of large myelinated fibres. Both of these findings correlate with GAA expansion size (Santoro *et al.* 1999). It is thought that the sensory neuropathy in FRDA results from a combination of inefficient myelination and superimposed slowly progressive axonopathy (Koeppen & Mazurkiewicz 2013, Morral et al. 2010).

Abnormalities of eye movement are a common early sign in FRDA. Probably the commonest feature is fixation instability interrupted by involuntary saccades, or square wave jerks (SWJs), which can occur in primary position, horizontal or vertical fixation (Furman *et al.* 1983, Fahey *et al.* 2008, Schöls et al. 1997). Square wave jerks can also interrupt smooth pursuit movements and be so prominent that they inhibit assessment of smooth pursuit and nystagmus. Nystagmus is less common but still frequent. It is typically horizontal gaze-evoked nystagmus on lateral gaze, and less commonly on vertical gaze (Harding 1981, Dürr et al. 1996, Schöls et al. 1997). Despite these commonly observed abnormalities, patients are usually only troubled by transient 'focusing' difficulties on gaze deviation. Fahey *et al.* (2008) found only 20% of patients had symptomatic oscillopsia.

Smooth pursuit movements typically have normal or only slightly reduced velocity, but are commonly interrupted by saccadic intrusions (Harding 1981, Dürr et al. 1996). Saccadic velocities are often normal but dysmetria is very common, often with a mix of hypo- and hyper-metria (Furman et al. 1983). Fahey *et al.* (2008) estimated that 54% of saccades were accurate to within 10% of the saccadic amplitude, 37% were hypermetric and 9% were hypometric. Ptosis is found in a small but significant proportion of cases, perhaps 5-10% (Filla et al. 1990, Schöls et al. 1997, Arnold *et al.* 2006).

Decreased visual acuity is less commonly seen than eye movement abnormalities and the majority of patients are asymptomatic. Approximately 20% of patients have decreased visual acuity (Harding 1981, Dürr et al. 1996, Fortuna *et al.* 2009) including occasional patients who have sudden bilateral loss of vision, mimicking Leber's hereditary optic atrophy. 30% of patients have disc pallor visible on fundoscopy (Harding 1981). In symptomatic patients, field loss may show generalized concentric field loss, concentric superior-inferior arcuate defects or isolated paracentral field loss. Despite the lack of symptoms, all patients show reduced retinal nerve fibre layer thickness throughout all four quadrants on optical coherence tomography (Fortuna et al. 2009). Pattern visual-evoked potentials show increased latency in 34-70% of patients (Carroll *et al.* 1980, Dürr et al. 1996, Fortuna et al. 2009). Apparent diffusion coefficients on diffusion-weighted magnetic resonance imaging of the optic radiations

were significantly higher than controls (Fortuna et al. 2009). Taken together, these findings suggest that the entirety of the visual system is involved in FRDA, although a minority of patients are clinically affected.

Dysarthria is a common and early sign present in more than 90% of individuals which progresses with disease duration. Speech becomes slow and slurred, impairing intelligibility in advanced cases. A study of 38 individuals with FRDA showed that 68% had mild dysarthria characterized by consonant imprecision, decreased pitch variation, impaired loudness maintenance, reduced phrase length, hypernasality and impaired breath support for speech (Folker *et al.* 2010). Cluster analysis revealed two further subgroups with increased laryngeal dysfunction (13%) and increased velopharyngeal involvement (11%).

Mild dysphagia is also a common symptom and can become problematical in advanced disease, occasionally requiring percutaneous endoscopic gastro-oesophageal tube insertion. Patients may cough or choke on solids or liquids including saliva. Dry dusty foods, small particulate foods such as peanuts or fruits which produce large amounts of juice on chewing are particularly difficult. Chewing may also be compromised requiring avoidance of tough foods or cutting of food into small pieces. Estimates of dysphagia frequency vary between 27 and 74% (Filla et al. 1990, Dürr et al. 1996, Schöls et al. 1997).

Hearing difficulties due to auditory neuropathy are a common and understated problem which can be very socially disabling even in the early stages of disease. Reported prevalences of hearing loss in case series vary widely from 8 to 39% (Geoffroy et al. 1976, Harding 1981, Dürr et al. 1996, Schöls et al. 1997) with Harding (1981) finding mild deafness in 5.2%, moderate in 1.7% and severe in 0.9%. Sound perception, as measured by hearing thresholds across audiometric frequencies in a quiet room (250-8000Hz) is typically normal in FRDA (Rance *et al.* 2008) or there may be minor deficiencies at different frequencies (Satya-Murti *et al.* 1980, López-Díaz-de-Leóna *et al.* 2003). However, almost all patients show disordered neural conduction in the central auditory pathways which functionally results in impaired speech understanding in conditions of background noise typical of everyday listening

conditions. This can lead patients to be able to access only 50% of information available compared to unaffected individuals (Rance *et al.* 2010). Peripheral auditory (outer and middle ear) function is generally unaffected, as shown by normal tympanometry and equal air and bone conduction in pure tone audiometry (Satya-Murti et al. 1980). Pre-neural cochlear responses, such as oto-acoustic emissions, are also normal (López-Díaz-de-Leóna et al. 2003). However, retrocochlear and brainstem responses, such as acoustic reflexes, synthetic sentence identification with ipsilateral competing message and brainstem-evoked auditory potentials, are abnormal (Satya-Murti et al. 1980, Jabbari *et al.* 1983, López-Díaz-de-Leóna et al. 2003). In particular, there appears to be impairment of temporal resolution of complex acoustic signals as shown by temporal discrepancies between oto-acoustic emissions, cochlear microphonics and brainstem-evoked auditory potentials (Rance et al. 2008). Such auditory neuropathy dys-synchrony grossly impairs the ability to perceive rapidly changing auditory signals which is vital for phoneme discrimination and so speech perception.

Sphincter disturbance is poorly studied in FRDA but its prevalence is said to range from 7 to 41% in case series (Andermann et al. 1976, Filla et al. 1990, Dürr et al. 1996, Schöls et al. 1997, Delatycki et al. 1999). Urinary urgency with secondary urinary incontinence is the commonest problem encountered, with urodynamic studies showing uninhibited contractions and altered bladder capacity (Vezina *et al.* 1982, Nardulli *et al.* 1992). Such symptoms of bladder hyperactivity are common in FRDA and are functionally exacerbated by mobility and transfer problems; however, suprapubic or transurethral catheterization is rarely required. Bowel problems have not been systematically studied in FRDA but are generally less troublesome than urinary problems.

Although ‘decrease in I.Q.’ was specifically mentioned in the first clinical criteria for FRDA (Geoffroy et al. 1976), most early studies concluded that cognition was not affected other than slowed information processing (Corben *et al.* 2006). Assessment of cognitive function can be significantly hampered by motor, speech and auditory impairments influencing reaction times, fluency and comprehension. Clinicians’ general experience is that *cognitive* deficits do not impede participation in education, employment or social activities. Mantovan showed impairments in tasks related to

visuoconstructive and visuoperceptual capacity, verbal fluency and motor and mental reaction times (Mantovan *et al.* 2006). The intelligence profile of FRDA patients was characterized by concrete thinking and poor capacity in concept formation and visuospatial reasoning. De Nóbrega found that patients with FRDA performed significantly worse in tests of phonemic and action fluency but not semantic fluency, when compared to controls (De Nóbrega *et al.* 2007). They postulated that this might represent primary prefrontal or cerebello-prefrontal dysfunction. More recent studies provide evidence in the growing field of cerebellar cognitive function and suggest that interruptions of the cerebro-cerebellar circuits may be functionally important in FRDA (Corben *et al.* 2010, Corben *et al.* 2011a, Corben *et al.* 2011b, Corben *et al.* 2011c, Klopper *et al.* 2011, Neito *et al.* 2012).

A variety of other features have been reported in the presence of FRDA although not necessarily related. As with all chronic disorders, depression is more prevalent than in the unaffected population. Flood and Perlman found that 92% of patients with FRDA showed an affective disorder ranging from mild mood disturbances to major depression (8%) (Flood & Perlman 1987). Epstein and colleugues found the Modified Fatigue Impact Scale was significantly worse in FRDA patients than controls. This can be a significant problem in some patients which often limits attempts at rehabilitation or ongoing exercises to maintain physical function (Epstein *et al.* 2008). Autonomic changes have gained little attention in the literature. Of these, a particularly troublesome and intractable symptom for a large number of patients is vasomotor disturbance, especially cold feet. Filla found vasomotor disturbance or hyperhydrosis of the extremities in 48% of patients (Filla et al. 1990).

1.4.2 Non-Neurological Features

Evidence of cardiac complications is found if sought in probably the majority of cases of FRDA although the patients are very often asymptomatic. Palpitations are sometimes reported but overt symptoms of heart failure are uncommon. It is rare for cardiomyopathy to develop before neurological features, and even if the patient is initially referred to a cardiologist, on detailed history or examination, neurological features are usually found which preceded the cardiac complications. Ischaemic heart

disease is also rare. In large cases series, hypertrophic cardiomyopathy or evidence of left ventricular hypertrophy (LVH) was found in 28-100% (Geoffroy et al. 1976, Ackroyd *et al.* 1984, Filla et al. 1990, Dürr et al. 1996, Schöls et al. 1997, Delatycki et al. 1999, McCabe *et al.* 2000) although definitions vary widely between studies. Asymmetric septal hypertrophy or dilated cardiomyopathy is less commonly seen and may represent progression from hypertrophic cardiomyopathy (Casazza & Morpurgo 1996). Blood pressure is typically normal or low, and hypertension is rarely a problem. Absence of correlation between the presence of cardiac complications and severity of neurological involvement has been reported (Weidemann *et al.* 2012). The disjunction between cardiac and neurological features may result from tissue-specific somatic instability and mosaicism of the GAA expansion.

The electrocardiogram (ECG) is abnormal in almost all cases, the commonest anomaly being inferolateral or widespread T-wave inversion. Other non-specific ST segment and T-wave abnormalities, including ST-segment depression or elevation and flattening of T waves, are also seen (Dutka *et al.* 1999, Schadt *et al.* 2012). ECG evidence of LVH is seen less frequently and if present is usually accompanied by echocardiographic evidence of LVH. QRS axis deviation is variable but most commonly to the right (Dutka et al. 1999, Weidemann et al. 2012, Kipps *et al.* 2008, Schadt et al. 2012). Conduction abnormalities are very rare (Weidemann et al. 2012, Schadt et al. 2012). Sinus rhythm or sinus tachycardia is usually found, although patients may be troubled with paroxysmal or sustained arrhythmias, particularly atrial fibrillation, and only rarely require pacemaker or defibrillator insertion (Bourke & Keane 2011).

Echocardiographic studies again show very variable results between patients. LVH is usually seen which is most commonly concentric but can show asymmetric septal hypertrophy (Dutka et al. 1999). There is impaired systolic function but with relatively preserved ejection fraction. In a longitudinal study including 113 echocardiograms in children, median ejection fraction was 61% (Kipps et al. 2008). Systolic function shows a slow non-linear decline with ejection fraction decreasing more rapidly with increasing age (Kipps et al. 2008, Regner *et al.* 2012).The cardiac valves are generally normal but hypertrophied papillary muscle may be seen (Dutka et al. 1999). In a large study of 204 patients with FRDA, 140 (69%) had evidence of cardiomyopathy, of which

58.5% were classified as mild, 23.5% intermediate and 18% severe. The mean interventricular septal thickness at diastole across these groups was 12.0mm, whilst left ventricular posterior wall thickness at diastole was 10.8mm and ejection fraction 63.2% (Weidemann et al. 2012). A study of 173 patients showed evidence of diastolic dysfunction in 84% of cases with pseudonormalization and impaired relaxation being the commonest descriptions (Regner et al. 2012). Cardiac magnetic resonance imaging studies have broadly confirmed the echocardiographic studies (Weidemann et al. 2012, Meyer *et al.* 2007) with increased left ventricular mass seen in FRDA, especially with short disease duration and greater GAA size. There seems to be a tendency to left ventricular thinning with longer disease duration (Rajagopalan *et al.* 2010).

The association between FRDA and diabetes mellitus, although suspected for many years, was only confirmed relatively late (Thorén 1962, Hewer & Robinson 1968). The mechanism of this is unclear but may relate to a combination of both insulin resistance of peripheral tissues such as muscle, and also decreased insulin secretion resulting from pancreatic beta cell dysfunction (Finocchiaro *et al.* 1988). These abnormalities in turn are likely to result from mitochondrial dysfunction. There does not appear to be an underlying immune pathology driving these changes (Schoenle *et al.* 1989). There is some evidence that heterozygous carriers of the GAA expansion in the frataxin gene may have increased incidence of insulin resistance (Fantus *et al.* 1991, Hebinck *et al.* 2000). In case series, diabetes mellitus was found in 6-19% of cases (Andermann et al. 1976, Harding 1981, Filla et al. 1990, Schöls et al. 1997, Delatycki et al. 1999, McCabe et al. 2000).

Scoliosis is common although may be mild and not require surgery especially if disease onset is relatively late. Scoliosis is a relatively common initial presentation of FRDA particularly when there is poor recovery from scoliosis surgery requiring prolonged rehabilitation, or the presence of subtle neurological signs at or after surgery, both of which may warrant further neurological investigations which uncover the underlying diagnosis. Labelle and coworkers found scoliosis of more than 10 degrees in 100% of patients and hyperkyphosis in 66% (Labelle *et al.* 1986). Most cases showed double thoracic and lumbar curves. Of those that were followed up long-term, roughly equal numbers progressed or were non-progressive. Milbrandt and colleagues in a series of

77 patients found that 63% had scoliosis and 24.5% hyperkyphosis. 33% had a double major curve (Milbrandt *et al.* 2008). 20% were treated with braces and 33% underwent spinal fusion. In other case series, 33 to 94% of patients had scolioisis, most series finding a prevalence of more than 75% (Geoffroy et al. 1976, Harding 1981, Ackroyd et al. 1984, Filla et al. 1990, Dürr et al. 1996, Schöls et al. 1997, Delatycki et al. 1999, McCabe et al. 2000).

Foot abnormalities are common. Case series show foot deformities in 55 to 90% of cases (Geoffroy et al. 1976, Harding 1981, Ackroyd et al. 1984, Filla et al. 1990, Dürr et al. 1996, Schöls et al. 1997, Delatycki et al. 1999, McCabe et al. 2000). Friedreich observed that both pes cavus and talipes equinovarus occur, either singly or in combination. Although more recent literature has concentrated on pes cavus, talipes equinovarus is probably more common. Pes planus is sometimes also seen (Harding 1984). Talipes equinovarus is a progressive condition found in advanced disease and can be very disabling to mobility, transfers and seating. If the patient is still ambulant, it can prevent proper placement of the foot on the floor and so contribute to instability and requirement for walking aids or orthotic devices. If the patient is wheelchair-bound, it can impede positioning and transfers (Delatycki *et al.* 2005). It can therefore increase carer demand and affect independence and quality of life.

1.4.3 Progression and Mortality

There is no accepted measure of progression in FRDA and a variety of rating scales and performance measures have been employed which include combinations of neurological and other parameters, or more specific measures of particular signs or symptoms. At a more basic level, Harding found that 72% of patients in her series were wheelchair-bound (Harding 1981). The mean duration from age at onset to age at wheelchair-bound was 15.5 years (range 3-44) which represented a mean age of 25 (range 11-58). The distribution of age at wheelchair-bound was not parametric with a prolonged upper tail. Half of cases were wheelchair-bound 16 years after onset of symptoms (age 26 years) and 95% 23 years after onset (age 44). Various clinical parameters progressed at different rates, with dysarthria present in 100% of cases by 10-15 years since onset, LL pyramidal weakness by 25-30 years since onset and distal

UL wasting and loss of vibrational and joint position sense by 45-50 years since onset. Schöls and coworkers found the mean time from onset to wheelchair-bound to be 11 years (range 5-26) representing a mean age of 24 (range 15-44) (Schöls et al. 1997). Mean age at onset of dysarthria was 20.8 years in a group whose mean age at onset of any symptom was 14.2 years. These measures correlated with the length of the shorter GAA expansion. Mean age at onset of UL ataxia was 19.8 years. De Michele and colleagues found the median time to loss of independent gait was 8 years (range 2-25), and to wheelchair-bound 15 years (range 6-29) (De Michele *et al.* 1996). Symptom onset before 20 years of age and the presence of LVH, predicted increased rate of progression. 17% developed diabetes with a median time from symptom onset of 16 years (range 4-27) corresponding to a median age of 29 (range 9-42).

Fahey and colleagues studied different rating scales to determine which best captured disease progression in FRDA (Fahey *et al.* 2007), comparing the Friedreich's Ataxia Rating Scale (FARS) (Subramony *et al.* 2005), the International Co-operative Ataxia Rating Scale (ICARS) (Trouillas *et al.* 1997) and other less specific measures. They found a mean change of 9.5 points in the FARS and 5 points in the ICARS over 12 months. They suggested the FARS required fewer patients for an equivalently powered study, although the ICARS requires less time to administer (Schulz *et al.* 2009). Friedman and coworkers studied 236 patients with FRDA, 159 of whom returned for follow-up after 1 year and 124 after 2 years (Friedman *et al.* 2010). They used a variety of clinical rating scales and performance measures to monitor disease progression including those relating to specific symptoms (9-hole peg test, timed 25-foot walk, PATA speech test, low-contrast letter acuity vision charts) and more general scales (FARS, functional disability scale, activities of daily living). These measures and various composite versions captured disease progression although with differing sensitivities, linearity and subjection to bias, ceiling and floor effects. In particular, they noted that the results of the 9-hole peg test changed linearly over time, whereas the timed 25-foot walk and the low-contrast letter acuity test were more susceptible to floor and ceiling effects caused by asymptomatic and maximally affected individuals respectively. The PATA speech test did not change over time making it unrepresentative of disease progression. The FARS and a composite measure called Z3 (combining the 9-hole peg

test, the timed 25-foot walk and the low-contrast acuity test) captured change in both ambulatory and non-ambulatory patients, whereas a more restricted composite measure (Z2) combining only the 9-hole peg test and the timed 25-foot walk was more liable to ceiling effect.

The same group in an expanded cohort of 259 patients found the annual rate of change of the FARS to be 2.66 points over the first year and 6.20 points over the first two years of follow-up. Assessment over the subsequent four years showed marked ceiling effect, as demonstrated by a diminution in the annual rate of change of the FARS for those with a baseline FARS of greater than 89 (ie those with more severe ataxia). The feature was particularly apparent in those with GAA repeat sizes of greater than 600 (Regner et al. 2012).

Metz and colleagues carried out a cross-sectional study which provides a detailed characterization of the ICARS in 603 FRDA patients (Metz *et al.* 2013). Interestingly the analysis showed different rates of progression for patients with early and late disease onset, 2.5 ± 0.18 points and 1.8 ± 0.27 points per year respectively. It is noteworthy for future trials that ceiling effects in the posture, gait and lower limb scale items lead to reduced sensitivity of the scale in the severely affected population with a total score of >60 points. Bürk and coworkers rated 96 patients using three different clinical scales, the FARS, ICARS, and the Scale for the Assessment and Rating of Ataxia (SARA) (Bürk *et al.* 2009). Although these rating scales have very different structures from each other, total SARA scores were significantly correlated with ICARS and FARS making the SARA, which is shorter and quicker, suited for trials. However a ceiling effect is also seen in the use of the SARA in patients in late stages of the disease.

Cardiac complications are the commonest cause of death in FRDA. Andermann found mean age at death of 30.6 years (range 4.5 to 40) in the 15 patients who died as part of their study of 58 Canadian patients (Andermann et al. 1976). Leone found median age at death of 34.5 (range 19-54) amongst the 14 patients who died in their cohort of 59 North-west Italian patients (Leone *et al.* 1988). Recorded causes of death included diabetic coma, myocardial infarction, bronchopneumonia and wheelchair accident. Females had better prognosis than males. In a further Italian study, De Michelle found

median age at death of 41 (range 10-65) amongst 11 patient who died in their study of 119 individuals (De Michele et al. 1996). The presence of diabetes and left ventricular hypertrophy decreased survival time. The largest study of mortality in FRDA looked retrospectively at the notes of 61 individuals who had died. Mean age at death was 36.5 years (range 12-87) with cardiac or probable cardiac dysfunction accounting for 62% of cases (Tsou *et al.* 2011). Of these, the majority resulted from congestive cardiac failure or arrhythmia. Other causes of death included stroke, ischaemic heart disease and pneumonia. Increased GAA expansion size, presence of arrhythmia and dilated cardiomyopathy were greater in deceased compared to live patients, but there was no difference in hypertrophic cardiomyopathy.

1.4.4 Clinical Subtypes: Early & Late Onset Disease

Since the discovery of the genetic mechanism underlying FRDA, cases with later onset, usually with milder or less typical symptoms, slower progression and a lower prevalence of non-neurological features, have been described, forming the majority of atypical cases. Onset may be in the sixth or seventh decades of life in which case symptoms may be mistaken for alternative diagnoses (Berciano *et al.* 2005, Galimanis *et al.* 2008). It is now felt that up to 25% of patients do not fulfil the original clinical criteria proposed by Geoffroy or Harding (Harding 1981, Dürr et al. 1996, Schöls et al. 1997, Filla *et al.* 2000, Barbeau 1984, Geoffroy et al. 1976). In the literature there are two main late-onset atypical presentations of FRDA described: (i) late-onset FRDA (LOFA) or very late onset FRDA (VLOFA); and (ii) FRDA with retained reflexes (FARR) (Klockgether *et al.* 1996, Coppola *et al.* 1999, Verma & Gupta 2012). LOFA and VLOFA are defined as FRDA with onset after the age of 25 years and after 40 years respectively.

Gait and limb ataxia are the presenting clinical features in several series of LOFA (Bhidayasiri et al. 2005, Montermini et al. 1997, De Michele *et al.* 1994, Gellera *et al.* 1997, Ragno *et al.* 1997, Klockgether *et al.* 1993, Coppola et al. 1999). Dysarthria remains a consistent feature, but is found later in the course of disease and correlates with disease duration. Pyramidal involvement with or without increased tendon reflexes or muscle tone is considerably less prominent in LOFA. The presence of

spasticity in LOFA ranges between 30 and 40% but does not correspond to extensor plantar responses which are found in 40 to 100% of atypical cases. In VLOFA, spastic tetraparesis has been reported without marked ataxia (Labauge 2002, Lhatoo *et al.* 2001, Berciano *et al.* 2002). Data on the presence of lower limb neuropathy in LOFA patients is sparse. Bhidayasiri and colleagues reported only one out of 13 patients with atypical FRDA not having sensory neuropathy (Bhidayasiri et al. 2005). De Michele and coworkers reported abnormal peripheral sensory and motor neuropathy conduction studies in 16 atypical FRDA cases, mainly sensory axonal neuropathy (De Michele et al. 1994). Subtle sensory neuropathy, a hallmark of classical FRDA, has also been reported in VLOFA (Berciano et al. 2005, Lhatoo et al. 2001). Oculomotor abnormalities may be absent in LOFA.

Non-neurologic manifestations such as scoliosis, pes cavus, cardiomyopathy and diabetes are considerably less frequent in LOFA. While the presence of pes cavus varies between 33 and 45%, scoliosis may be subtle and present in less than 40% of cases (Dürr et al. 1996, Bhidayasiri et al. 2005, De Michele et al. 1994, Gellera et al. 1997, Coppola et al. 1999, Klockgether et al. 1993, Ragno et al. 1997, Schöls et al. 1997). Cardiomyopathy may be absent in the atypical phenotype. Two recent large studies on heart involvement in more than 350 FRDA patients revealed no cardiomyopathy in up to 40% of patients and a correlation of cardiac abnormalities with GAA1 (Weidemann et al. 2012, Regner et al. 2012). Abnormal findings on echocardiogram in atypical FRDA range between 0 and 57% (Schöls et al. 1997, Dürr et al. 1996, Gellera et al. 1997, De Michele et al. 1994, Bhidayasiri et al. 2005, Coppola et al. 1999) Abnormal electrocardiogram with T-wave inversion, left axis deviation, and repolarization abnormalities, however, is found in almost all FRDA patients.

The ability to measure *FXN* gene dosage levels, frataxin mRNA expression levels and frataxin protein levels seems likely to allow the identification of further atypical cases which are not diagnosed by current genetic techniques. In one study, mean residual levels of frataxin were 65.6% in LOFA and 35.8% in classical FRDA, compared to 100% in controls, with a direct correlation between frataxin protein levels, age at onset and GAA repeat size (Saccà et al. 2011). Messenger RNA expression in LOFA has been shown to have reduced levels, from 20 to 50% of controls (Pianese et al. 2004, Saccà et

al. 2011). Surprisingly however, the latter study showed that frataxin protein and mRNA expression levels overlapped in LOFA patients, controls and carriers (Saccà et al. 2011). The measurements were performed on peripheral blood mononuclear cells which may not be representative of affected tissues because of somatic mosaicism or mitotic instability, but it may be that there are as yet undiscovered determinants of disease in FRDA other than frataxin protein and mRNA levels, such as genetic modifiers outside the *FXN* gene.

Early-onset cases have been little studied. Harding found that less than 20% of cases had onset before the age of 5 (Harding 1981). She commented that patients with onset before the age of 10 who deteriorate rapidly are often short in stature and have short limbs, unless they are ambulant throughout the growth period (Harding 1984). Kinnear Wilson was struck by 'by the general undersized, almost infantile physique of a Friedreich patient.' (Wilson 1940). Early age at onset is associated with larger GAA1 size (Dürr et al. 1996, Schöls et al. 1997) which is associated with more severe phenotype, faster progression of disability and higher incidence of cardiomyopathy, pes cavus and scoliosis. Dürr *et al.* (1996) found that patients with GAA1 sizes of greater than 780 repeats had a mean age at onset of 9.7 years, compared to those with GAA1 of less than 520 repeats who had a mean age at onset of 22.5 years. More recently, the identification of exonic deletions in the frataxin gene has suggested that these may cause a more severe and earlier presentation, and may contribute more to the clinical picture of FRDA than is currently appreciated (Anheim *et al.* 2012). This subject is explored in Chapter 3.

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Chapter 2 : Natural History of Friedreich's Ataxia

2.1 Introduction

This chapter describes clinical data collected at the National Hospital for Neurology and Neurosurgery (NHNN) as part of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) natural history study of FRDA. The EFACTS consortium comprises 18 centres in 7 European Union (EU) member states, of which 11 are involved in the clinical project and 10 in basic sciences projects (see Figure 4). The aim of the clinical project was to recruit 600 patients with FRDA across Europe and assess them at yearly intervals over a four year period and beyond. Participating patients undergo a comprehensive clinical assessment including a panel of assessment tools allowing their validation in a large longitudinal cohort of clinically and genetically well-defined patients.

Each patient provides a blood and urine sample allowing the generation of a central biological sample repository. These samples are used to confirm the genetic diagnosis and permit further genetic and epigenetic characterization. Frataxin mRNA and protein expression will be determined on peripheral blood samples. Serum and plasma samples will be used for proteomic analysis. These measures will therefore allow the identification of novel biomarkers and genetic modifiers.

The clinical, genetic and biochemical results are entered into a central anonymized database, allowing data modelling and specialized computational and statistical analysis. These may permit the identification of new pathways to facilitate greater understanding of the underlying pathogenesis of FRDA and potential new drug targets. The validation of clinical assessment tools will enable their use in clinical trials and other studies, and provide essential data for power calculations for such studies. More widely, the EFACTS project also encompasses basic scientific studies on frataxin structure and function, genetic and epigenetic mechanisms, cellular and animal models, and therapeutic trials.

Figure 4: Clinical centres (blue) and basic science centres (red) participating in EFACTS

2.2 Method

2.2.1 Ethics, Recruitment & Funding

The project was approved by the Central London Research Ethics Committee (reference 10/H0716/51) and is funded by a Framework Project 7 grant from the EU (reference HEALTH-F2-2010-242193). Patients with FRDA were identified from the records of the NHNN and contacted initially using a standard letter enclosing the Participant Information Sheet (PIS). Telephone contact was subsequently made if necessary, or the project was discussed during the patients' routine appointment at the NHNN. An advertisement was also placed in The Ataxian – the newsletter of Ataxia UK – which is circulated to members of Ataxia UK, the UK's leading charity concerned with all forms of ataxia, as well as being available on their website (www.ataxia.org.uk). A further advertisement was placed in the electronic newsletter of the Association of British Neurologists (ABN) which is read by members of that

organization who are primarily neurologists practising in the UK. In addition, the website of EFACTS (www.e-facts.eu) contains contact details by which patients can contact the NHNN research group directly, and a facility for patients to obtain further information about the project centrally and if appropriate be put in contact with the local research groups. Further patients were identified via Ataxia Ireland (formerly the Friedreich's Ataxia Society of Ireland) who also provided funding for the travel expenses of patients. The establishment of a Participant Identification Centre (PIC) at the Ataxia Centre of the Royal Hallamshire Hospital, Sheffield (Prof. Mario Hadjivassiliou) enabled the recruitment of a further tranche of patients.

2.2.2 Clinical Assessment

Patients were invited to attend a research clinic at the NHNN. Each patient provided signed informed consent after having received the PIS. Travel expenses were paid from the EFACTS funding. A small number of patients were seen as part of home visits. The clinical assessment lasted approximately two hours. Each patient provided basic demographic details including country of birth, ethnic origin, level of educational achievement, occupational history, marital and family status. Education was graded according to the International Standard Classification of Education (ISCED) formulated by the United Nations Educational, Scientific and Cultural Organization (UNESCO) to ensure comparability between different countries.

A structured history of the disease was elicited including age and nature of first symptoms, date of clinical and genetic diagnosis, and significant disability milestones such as age of first falls, and intermittent or permanent use of aids to mobility or wheelchair. Details of associated symptoms were recorded such as visual impairment, hearing loss, cardiac involvement, scoliosis and pes cavus. A detailed family history was recorded including the relation, age, age at onset and age at death of family members diagnosed with FRDA and other causes of ataxia. A detailed structured past medical history was recorded. A detailed history was recorded of present and past use of medications, vitamins, supplements, alcohol, smoking and recreational drugs, as well as involvement in current or previous clinical trials.

Patients were requested to bring details of their most recent cardiac investigations including echocardiogram and electrocardiogram (ECG) and if these were not available, consent was obtained to request these from their cardiologist or General Practitioner. Three parameters were recorded from the echocardiogram, namely interventricular septal thickness at diastole (IVSd), left ventricular posterior wall thickness at diastole (LVPWd) and left ventricular ejection fraction (EF). These data were therefore not measured prospectively as part of the study and were undertaken in multiple centres by many different operators. The measurements were often not taken at the same time as the baseline clinic visit. In the case of echocardiograms, the data were obtained from the echocardiogram report or clinic letter. Because of the different protocols used in different centres, and because echocardiography is often technically difficult in wheelchair-bound patients for reasons of positioning, incomplete data sets were often found. Where a range of values was quoted for ejection fraction (*eg* 40-50%), the median value was used (*ie* 45%). In the case or ECGs, the original recording was usually acquired and directly assessed, although in a number of cases, the results were obtained from clinic letters. The latter practice may serve to overestimate abnormalities, as abnormal results are more commonly recorded in clinic letters than normal results. Where the ECG was directly scrutinized, it was adjudged to show voltage criteria of left ventricular hypertrophy (LVH) if the R wave in leads V_5 or V_6 exceeded 26mm or the sum of the R wave in V_6 and the S wave in V_1 exceeded 35mm (Sokolow & Lyon 1949). Blood pressure, pulse rate, weight and height were measured and recorded at each assessment.

Functional disability was assessed using the 9-field **Activities of Daily Living (ADL)** section of the Friedreich's Ataxia Rating Scale (FARS) (Subramony *et al.* 2005) which has previously been validated for use in FRDA (Lynch *et al.* 2006, Friedman *et al.* 2010). This covers speech, swallowing, use of cutlery, dressing, washing, falls, walking, sitting and bladder function, giving a maximum score of 36 points (thus, higher scores represent greater disability). Functional disability was further assessed using the 7-point **Spinocerebellar Degeneration Functional Score (SDFS)** which has been used in FRDA previously (Anheim *et al.* 2012). This is a 7-point scale in which higher scores represent greater disability, as follows:

1. No functional handicap but signs at examination
2. Mild, able to run, walking unlimited
3. Moderate, unable to run, limited walking without aid
4. Severe, walking with one stick
5. Walking with two sticks
6. Unable to walk, requiring wheelchair
7. Confined to bed.

Quality of life and health status were assessed using the **EuroQoL 5-dimension (EQ-5D)** scale (EuroQol-Group 1990). This is a self-reported questionnaire covering mobility, self-care, usual activities (work, study, housework, family or leisure activities), pain/discomfort and anxiety/depression. Higher scores represent greater disability or disease involvement. In addition, there is a visual-analogue scale (VAS ; the 'thermometer') rating health state on a scale of 0 to 100, in which 0 represents the worst imaginable health state and 100 represents the best imaginable heath state. There are also background questions covering demographics, previous experience of serious illness, employment, education and smoking. This has been validated in FRDA although the study found limited discriminant ability and responsiveness to progression (Riazi *et al.* 2006). There were also high levels of missing data particularly in the section on mobility.

Ataxic signs were assessed using the 8-field Scale for the **Assessment and Rating of Ataxia (SARA)** which covers gait, stance, sitting, speech, finger chase, nose-finger test, fast alternating hand movements and the heel-shin test. The scale gives a score from zero (no ataxia) to a maximum of 40 and has been validated in groups of patients with spinocerebellar ataxias (Schmitz-Hübsch *et al.* 2006), mixed ataxias (Weyer *et al.* 2007) and FRDA (Bürk *et al.* 2009).

Ataxic signs were further assessed by two performance measures, the **Spinocerebellar Ataxia Functional Index (SCAFI)** and the **Composite Cerebellar Functional Severity Score (CCFS)**. The latter is not presented in this thesis. The SCAFI includes the three functional performance measures taken with modifications from the FARS (Subramony *et al.* 2005) which has been validated in the spinocerebellar ataxias (Schmitz-Hübsch *et al.* 2008). The index assesses gait, manual dexterity and speech using an 8-metre timed

walk (8mTW) with or without assistive devices; the time taken to place and retrieve nine pegs in nine holes for each hand using the Rolyan plastic one-piece 9-hole peg test apparatus (9-hole peg test, 9HPT); and the number of vocalized repetitions of the two syllables 'pa-ta' in 10 seconds (PATA test). Higher scores on the 8mTW and 9HPT (*ie* the patient takes longer to undertake each task) and lower scores on the PATA test (*ie* the patient utters fewer repetitions in 10 seconds) represent greater disability. Each test is performed twice. The raw data can be used and a Z score calculated as follows:

$$\mathrm{Z}\ score = \frac{(average\ of\ both\ trials) - (mean\ of\ study\ population)}{(standard\ deviation\ of\ study\ population)}$$

The reciprocal of the 8mTW and 9HPT results are used so that greater values always represent better function. The maximal allowable values of the 8mTW and 9HPT are set at 180s and 300s respectively. In order to calculate the Z score, if a patient is unable to perform a test for reasons of physical disability, the values are set at 10 times the maximal value for the 8mTW and 9HPT (ie 1800s and 3000s respectively), and zero for the PATA test.

Non-ataxic signs were assessed using the **Inventory of Non-Ataxic Symptoms (INAS).** The INAS was first described for use in the SCAs for assessing additional non-ataxic symptoms (Jacobi *et al.* 2013) and subsequently used as part of the EUROSCA natural history study of SCAs 1,2,3 & 6 (Jacobi *et al.* 2015). The INAS assesses by examination the presence, absence or severity of a number of non-ataxic signs including spasticity, paresis, muscle atrophy, fasciculations, myoclonus, rigidity, chorea/dyskinesia, dystonia and resting tremor. These are assessed using a semi-quantitative scale (none/mild/moderate/severe). Reflexes are assessed as normal, hyperreflexic or areflexic, and extensor plantar reflexes as absent, unilateral or bilateral. Vibration sense is assessed using a Rydel-Seiffer graduated tuning fork at the external malleolus of the ankle (Whitton *et al.* 2005). A range of ophthalmological features are assessed as either present or absent (pursuit, square wave jerks, nystagmus, ophthalmoparesis, saccades, visual acuity). The INAS further assesses the presence, absence, severity or frequency of a number of non-ataxic symptoms as reported by the patient, including diplopia, dysphagia, urinary dysfunction, cognitive impairment, vertigo, speech

problems, problems with handwriting and muscle cramps which are assessed using a similar semi-quantitative scale. Thirty-three separate items are assessed providing a large amount of directly analyzable data relating to patients' symptoms and signs. The extracerebellar items are grouped into 16 areas. The presence or absence of signs in each area is scored as zero or one, and the total of these provides the INAS count ranging from zero (no non-ataxic signs) to a maximum of 16. Both in the raw data for the INAS and the INAS count, higher values represent greater disability.

All patients in the study underwent a thorough neurological examination at every presentation, but not all details of this were captured in the validated rating scales and other data recorded as part of the EFACTS assessment. Details of the remainder of the neurological examination were therefore recorded in the **Structured Neurological Examination (SNE).** This is a tool devised by the author as a formal record of the neurological examination using a variety of traditional bedside assessment techniques and semi-quantitative scales. These data were therefore not recorded centrally as part of the EFACTS Registry. Amongst the cranial nerve examination, the presence, absence and nature of the following features was recorded: pupillary abnormalities, ptosis, ophthalmoparesis, facial sensory loss, masticatory muscle weakness, facial weakness, hearing abnormalities, palatal movement abnormalities, sternocleidomastoid weakness, trapezius weakness, tongue atrophy, abnormalities of lingual tone. As part of the limb examination, the severity or extent of the following features was recorded: power, reflexes, sensation, muscle atrophy, tone. Power was evaluated using the modified Medical Research Council (MRC) scale (Guarantors-of-Brain 1986) as follows:

0. No contraction (MRC 0/5)
1. Flicker or trace of contraction (MRC 1/5)
2. Active movement with gravity eliminated (MRC 2/5)
3. Active movement against gravity (MRC 3/5)
4. Active movement against gravity and slight resistance (MRC 4-/5)
5. Active movement against gravity and moderate resistance (MRC 4/5)
6. Active movement against gravity and strong resistance (MRC 4+/5)
7. Normal power (MRC 5/5)

In order to present the results concisely, the individual power ratings were grouped as follows:

1. Upper limb proximal : shoulder abduction/adduction, elbow flexion/extension
2. Upper limb distal: wrist flexion/extension, finger flexion/extension, index finger abduction, little finger abduction, thumb abduction
3. Lower limb proximal: hip flexion/extension, Hip abduction/adduction, knee flexion/extension
4. Lower limb distal: ankle flexion/extension, ankle inversion/eversion, toe flexion/extension

Deep tendon reflexes (biceps, supinator, triceps, patellar, ankle) were recorded according to the following scale:

0. Absent (-)
1. Present with reinforcement only ([+])
2. Present but hyporeflexic (+)
3. Normal (++)
4. Hyperreflexic (+++)
5. Hyperreflexic with clonus (++++)

The plantar reflexes were recorded as being extensor, flexor, mute or withdrawal.

Muscle atrophy was recorded using the following scale:

0. No atrophy
1. Mild atrophy
2. Moderate atrophy
3. Severe atrophy

Muscle tone was recorded according to the following scale:

1. Highly flaccid
2. Flaccid
3. Normal
4. Spastic
5. Highly spastic

If other variants of muscle tone were present, these were recorded. Upper limb sensation (pin prick, joint position sense, vibration) was recorded by extent of involvement according to the following scale on the presumption that the sensory loss was length-dependent in nature and predominantly distal in distribution:

0. Normal
1. Fingertips only affected
2. Affected to knuckles
3. Affected to wrists
4. Affected to elbows
5. Affected to shoulders
6. Other (specifiy)

Lower limb sensation was recorded using the following scale:

0. Normal
1. Toes only affected
2. Affected to ankles
3. Affected to knees
4. Affected to hips
5. Affected to lower costal margin
6. Affected to sternum
7. Other (specify)

Various other features such as skeletal foot abnormalities were also recorded as part of the SNE.

The EFACTS clinical assessment proforma and copies of all rating scales can be found in the Appendix.

2.2.3 Data Handling

All EFACTS data were entered into a central database (the 'EFACTS Registry') managed by 2mt Software GmbH (Ulm, Germany) after generation of a 9-digit anonymous code, the key to which was stored in each participating centre. Only clinicians and approved data management personnel in each centre had access to patient-identifiable records and data. The data were recorded in separate fields (*eg* demographics, onset, examination, cardiology, ADL, EQ-5D, SARA, INAS, SCAFI, *etc.*). Once the data were entered into the EFACTS Registry and finalized at each centre, they were then formally 'signed off' and underwent a process of data monitoring during which they were deemed either 'under editing' or 'plausible'. If individual data were missing, these could be recorded as such. If whole fields were not undertaken and the data would never become available, these could be 'deactivated'. The data monitoring process

generated data queries which were addressed by each participating centre, in order to eliminate internal inconsistencies in the data, implausible and missing results, and potential errors of data entry. Once this process was complete, the data were said to be 'checked' and were available anonymously to all participating centres. Difficulties, problems and queries concerning the Registry and clinical assessment generally, were discussed at monthly web-conferences to which all participating centres and senior members of the EFACTS Steering Committee contributed. Subsequent statistical analysis was performed using Excel and SPSS.

2.2.4 Biological Samples & Genetic Analysis

At the baseline and first year follow-up visits, 70ml of whole blood was taken and a urine sample given. All samples were processed within 30 minutes of withdrawal from the patient. 10ml of whole blood was taken into EDTA tubes for DNA extraction, genetic analysis and GAA size determination. On processing, the samples were frozen at -20°C and subsequently transferred to a -80°C freezer for long-term storage. 2.5ml of whole blood was taken into a Paxgene tube for frataxin mRNA analysis, inverted ten times and stored upright at room temperature for 24 hours to ensure cell lysis. It was then transferred to a -20°C for 48 hours before transferring to a -80°C freezer for long-term storage. The genetic and frataxin expression samples were repeated at the second year follow-up visit. 57.5ml of whole blood was taken in a 2:2:1 ratio into serum, EDTA and citrate blood tubes, respectively. These were centrifuged at 1,600G for 15 minutes at room temperature and the resulting supernatant fluid transferred to a pre-cooled matrix box on wet ice so that 16ml of serum, 16ml of EDTA-derived plasma and 8ml of citrate-derived plasma were obtained in 1ml sample tubes. The urine sample was also transferred to the matrix box in 1ml sample tubes. The matrix box was immediately transferred to a -80°C freezer. The resulting four blood tubes and matrix box were labelled anonymously with individual 8-digit codes issued by the Aachen biobank which were logged on the central EFACTS Registry.

The processed frozen blood and urine samples were stored at -80°C and periodically shipped under dry ice by temperature-controlled courier in batches to collaborating centres in Europe. Analysis of the blood samples for GAA size estimation, *FXN* gene

sequencing and measurement of frataxin expression were undertaken by collaborators at the Université Libre de Bruxelles (Dr. Myriam Rai & Pr. Massimo Pandolfo). Genomic DNA from peripheral blood lymphocytes was extracted using standard procedures and GAA expansion size estimated according to previously published methods (Pandolfo 2006) using primers as previously described (Campuzano *et al.* 1996). In short, this involved PCR using primers flanking the GAA expansion. The PCR products were subjected to electrophoresis on a standard agarose gel and the band size estimated by comparison with a standard DNA size ladder. The number of triplet repeats (n) was calculated according to the following formula: n=(bp-457)/3 where bp represents the size of the PCR product in base pairs. The smaller band is by convention denoted GAA1, and the larger, GAA2. The GAA size data are used in this thesis.

Where the results of genetic analysis performed centrally as part of the EFACTS group were not available at the time of data analysis, it was then performed locally in the accredited Neurogenetics laboratory of the NHNN which used triplet-primed and long PCR techniques to confirm the presence of two GAA expansions (protocols available locally). These techniques did not provide data concerning the GAA expansion size. In fact, the majority of patients in the study (who were recruited from local clinics at the NHNN) already had a genetic diagnosis provided by these techniques which allowed corroboration of the results produced by the EFACTS collaborators. Central EFACTS data were not available largely for phase 2 patients who entered the project at a later stage than phase 1 patients (see Section 1.3.1 below for an explication). Frataxin mRNA levels were also not available at the time of preparation of this thesis. The remaining blood samples will be stored in a central biobank at the Universitätsklinikum Aachen (Prof. Jörg B Schulz) who will coordinate proteomic and biomarker analysis of these samples between different collaborating centres.

2.2.5 Author's Contribution

The project was conceived by a working party of European research groups headed by Pr. Massimo Pandolfo of the Université Libre de Bruxelles which put together a funding application to the Seventh Framework Programme of the European Commission in 2009. This was successful and provided funding from 1.5.2010 to 30.4.2015. The

specific components of the clinical assessment and plans for the ascertainment, storage and processing of biological samples were discussed and agreed at the inaugural meeting of EFACTS at the European Commission, Brussels on 3-4 May 2010. Local Ethics Committee approval was obtained by Dr Suran Nethisinghe & Dr Paola Giunti, although the author fully formulated a successful substantive amendment to the application in 2013. The author first participated in the project at the EFACTS Research Meeting, Brunel University on 25-26 November 2010 and joined full-time at UCL on 4 April 2011. The author actively participated in the monthly pan-European web-conferences which discussed recruitment and data entry, as well as identifying ongoing problems with the project, devising solutions and proposing new sub-projects.

Prospective participants were identified by the author from records held in the Department and from other sources. Participants were contacted by the author in writing, by email or by telephone and research clinic appointments made by the author. Clinic facilities, laboratory space and clinical materials were obtained by the author. Approximately 90% of the patients were seen and fully assessed by the author, the remainder being seen by Dr Paola Giunti. All home visits were undertaken by the author. At each assessment the history, examination and rating scales were undertaken by the author as well as obtaining blood and urine samples. The vast majority of these were processed in the laboratory by the author, with help from Dr Vittoria Ercolani and Susan Green. Genetic analysis was performed by Dr Myriam Rai of the Université Libre de Bruxelles. Participants from the Republic of Ireland were identified by Barbara Flynn and Annette Kelly of Ataxia Ireland. Travel for the patients to London, or for the author and Dr Giunti were organized by Ataxia Ireland and Sarah Green of DeNDRoN.

Follow-up appointments were organized by the author and by Selina Paul of DeNDRoN, with approximately 90% seen and fully assessed by the author; the remainder were seen by Dr Paola Giunti. Data entry was performed by a variety of individuals including the author, with all data queries answered by the author and overall responsibility for quality control of data undertaken by the author. Management of the data centrally in the EFACTS Registry was undertaken by Kathrin Fesodov *geb* Osterholt. All local data handling, downloading, processing and statistical

analysis were undertaken by the author. The author left the project full-time on 5 August 2014 but has continued to participate in various elements of the study since then. Further details of the author's contribution are given in the Acknowledgements.

2.3 Results

2.3.1 Recruitment

Recruitment across the 11 European sites began on 15 September 2010 and by 30 April 2013 the 600 patient recruitment target had been attained. Baseline data for 592 of these cases for which a genetic diagnosis and full clinical data were available, was published in 2015 including 149 cases from the UK and Ireland (Reetz *et al.* 2015). These patients constitute phase 1 of the study. Recruitment continued and by 1 August 2014, 637 patients had been recruited across Europe including 169 from the UK and Ireland. The additional participants constitute phase 2 of the study. Thus, this centre provided 26.5% of the European total (see Figure 5). The distinction between phases 1 and 2 of the project is largely artificial and determined by internal recruitment and publication deadlines within the project. The only differences between the two groups were the funding rewarded to recruiting centres and the nature of biological samples taken for the central biobank. The clinical assessment was identical and so for the purposes of this thesis, the two are combined. However, because the phase 2 patients were recruited later than the phase 1 patients, there are almost no follow-up data on the former as they had not reached their follow-up appointments by the time the data were collated for this thesis. These data continue to be collected within the group as the project is ongoing but are not included in this thesis.

Recruitment in the UK for the purposes of this thesis took place between 1 August 2011 and 1 August 2014 and included 21 cases from the Republic of Ireland. 169 patients were recruited in total, for two of which genetic analysis subsequently showed no GAA expansions, point mutations or macrodeletions within the *FXN* gene. They were therefore excluded from all subsequent analyses. These two patients had been given a clinical diagnosis of FRDA before the discovery of the *FXN* gene in 1996 and had never subsequently undergone genetic testing. They were informed of the

genetic result and offered further investigations in the Ataxia Clinic. In neither case has this revealed a genetic diagnosis.

Site	Number Recruited
London	169
Milan	138
Madrid	78
Paris	51
Innsbruck	43
Munich	44
Brussels	34
Aachen	33
Tübingen	22
Bonn	18
Marburg	7
TOTAL	637

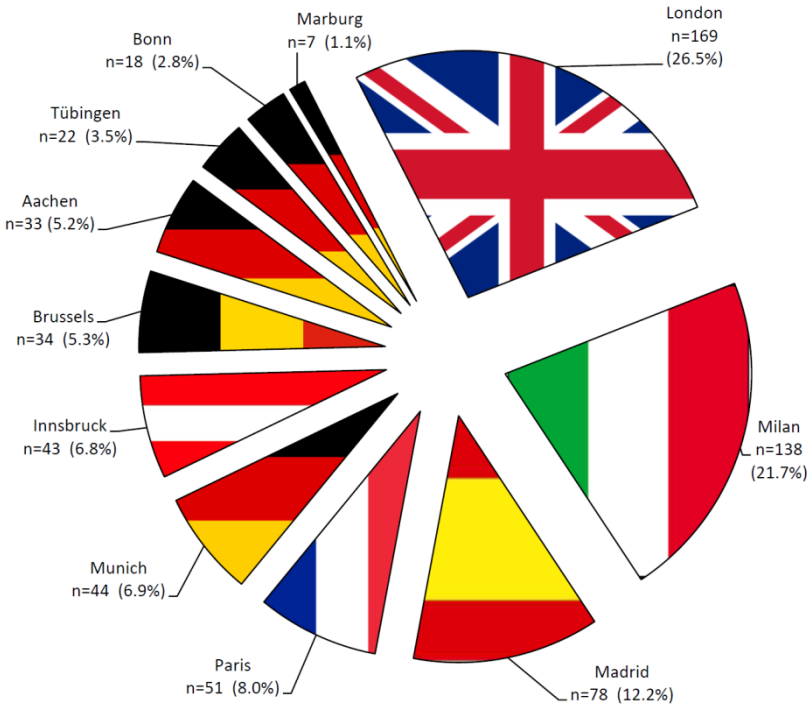


Figure 5: EFACTS patient recruitment by centre 15.9.2010-1.8.2014

2.3.2 Demographics

The basic demographic details at the baseline assessment of the 167 patients recruited at the London centre are given in Table 3, including gender, ethnicity, weight, height, country of birth, marital status, offspring, education and employment. Figure 6 and Figure 7 show the location of the patients, showing that, although the recruiting centre was in London, participants were recruited from throughout the UK and Ireland. Although 21 patients were recruited via the charity Ataxia Ireland, a further four were born in Ireland but recruited as part of the UK cohort (including one from Northern Ireland). The study included 15 pairs of siblings and 1 pair of cousins. 159 patients (95.2%) carried two GAA expansions. Of the remaining eight patients, all were compound heterozygotes. Seven had a point mutation, including five cases of the p.Gly130Val variant, and one each of the p.Met1Thr and p.Arg165Asp variants. There was one example of a macrodeletion. The compound heterozygotes are discussed more extensively in Chapter 3.

Table 3: Basic demographic details of UK EFACTS patients at baseline

		Number (%)
Gender	Male : Female	72[a] : 95 (43.1 : 56.9)
Age (years)	Range	16 – 68
	Mean ± SD	34.3 ± 13.2
	Median	31
Disease duration	Range	3 – 55
(years)	Mean ± SD	20.5 ± 11.2
	Median	19
Weight (kg)[b]	Mean ± SD	68.7 ± 16.1
	Range	40 – 120
Height (cm)[c]	Mean ± SD	168.2 ± 10.6
	Range	128 – 196
BMI (kg/m^2)[d]	Mean ± SD	24.3 ± 5.0
	Range	15.7 – 40.2
Ethnicity	Caucasian	155 (92.8)
	Asian	9 (5.4)
	S American	2 (1.2)
	Mixed	1 (0.6)
Country of birth	UK	125 (74.8)
	Eire	25 (15.0)
	South Africa	3 (1.8)
	Afghanistan	2 (1.2)
	France	2 (1.2)
	Italy	2 (1.2)
	Other[e]	9 (5.4)
Marital status	Single	106 (63.5)
	Married/in relationship	52 (31.1)
	Divorced/separated/widowed	9 (5.4)
Offspring	Children	47 (28.1)
	No children	120 (71.9)
Education (ISCED)[f]	1 : Primary school	1 (0.6)
	2 : 'O' level/GCSE	40 (24.0)
	3 : 'A' level	39 (23.3)
	4 : BTEC/NVQ/HND	32 (19.2)
	5 : BSc/MSc	48 (28.7)
	6 : PhD	6 (3.6)
	Not known	3 (1.8)
Employment	Not in employment	111 (66.5)
	In employment	56 (33.5)
	Full time	32 (19.2)
	Part time	24 (14.4)
Sibling pairs		15
Cousin pairs		1
Mutation	Homozygous GAA expansion	159 (95.2)
	Compound heterozygous[g]	8 (4.8)

[a] Includes 1 pre-operative transsexual living as female

cont…

[b]*n*=145; [c]*n*=150; [d]*n*=140 (incomplete dataset due to immobility)
[e]Canada, Colombia, Mauritius, New Zealand, Norway, Pakistan, Switzerland, Turkey, USA (1 each)
[f]ISCED=International Standard Classification of Education
[g]G130V (5), M1T (1), R165D (1), macrodeletion (1)

Figure 6: Location of EFACTS patients in the British Isles

Red dots indicate the current residence of patients participating in the study, showing their spread throughout the British Isles but concentration in the South-East of England

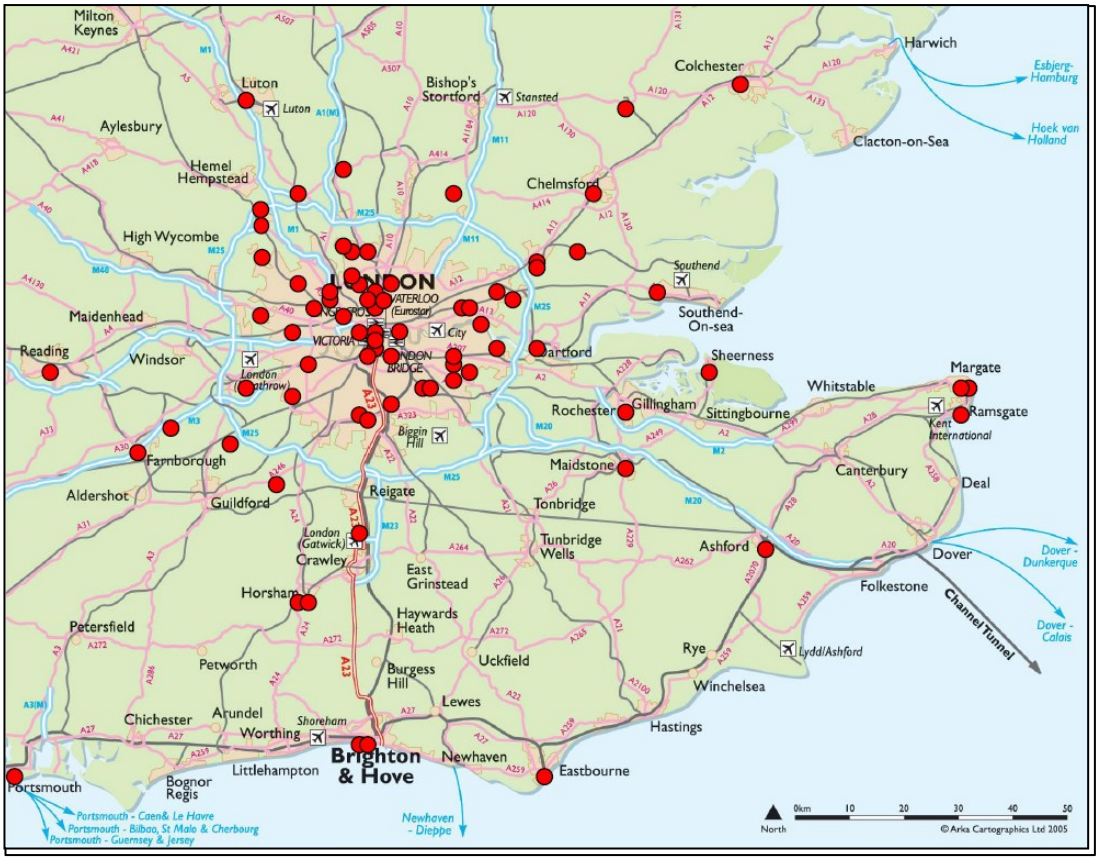


Figure 7: Location of EFACTS patients in the South-East of England
Red dots indicate the current residence of patients participating in the study from the South-East of England

The range of ages at which patients were examined at baseline as part of the study is illustrated in Figure 8 ranging from 16 to 68. Of note, only patients of 16 years and older were permitted by the local ethics permission, and so younger patients were not studied as part of this project. Figure 9 shows the disease duration since symptom onset for the EFACTS patients at their baseline visit, ranging from 3 to 55 years.

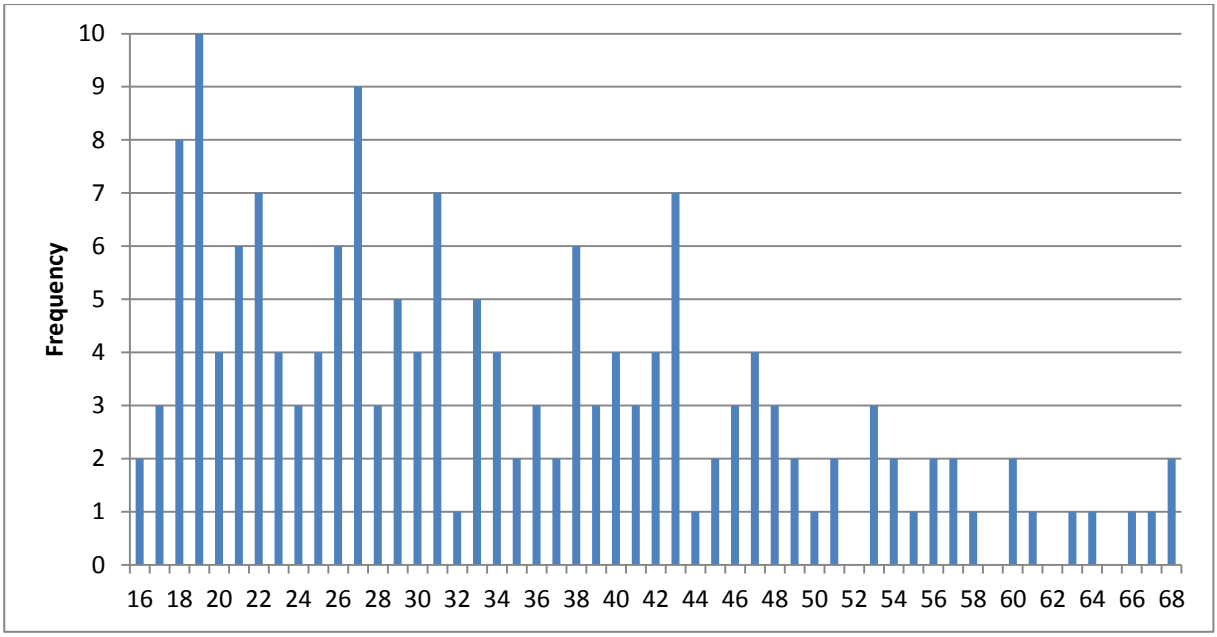


Figure 8: Age at examination of EFACTS patients at baseline

Patients were seen in the adult clinic from age 16 upwards. The oldest patient was 68 at baseline examination. The commonest age at baseline examination was 18, with the majority of patients below the age of 45

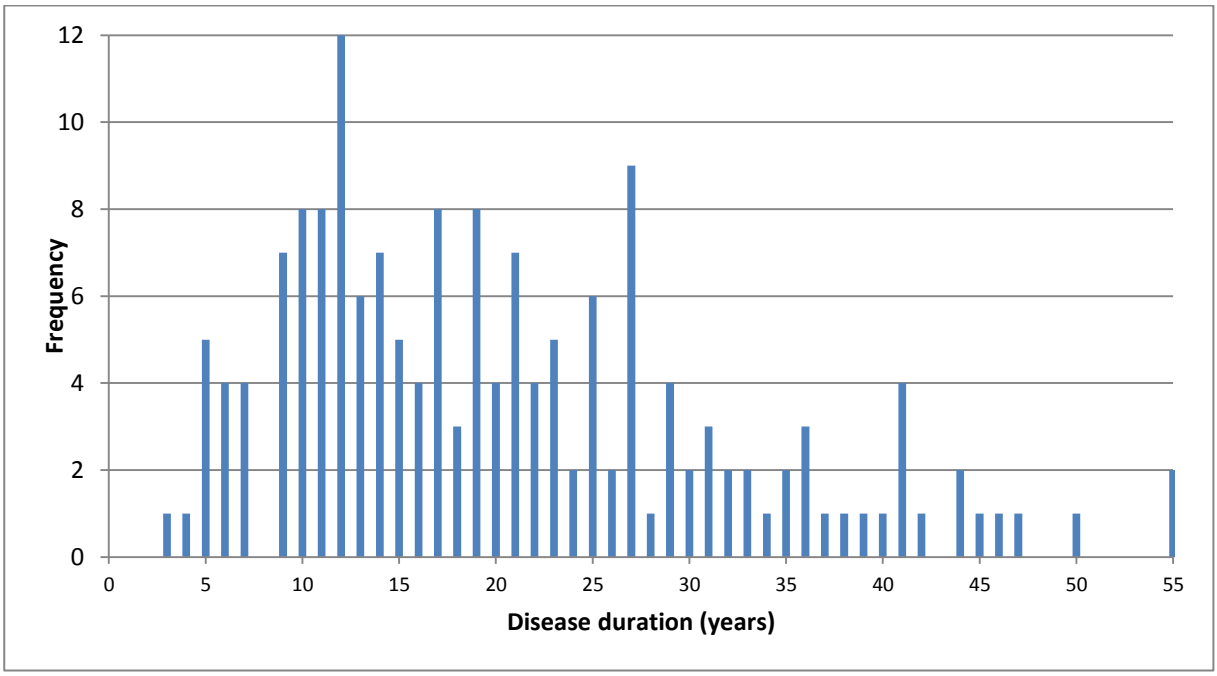


Figure 9: Disease duration of EFACTS patients at baseline

Patients seen at baseline examination had disease duration of between 3 and 55 years, with the commonest duration of 12 years and the majority less than 30 years

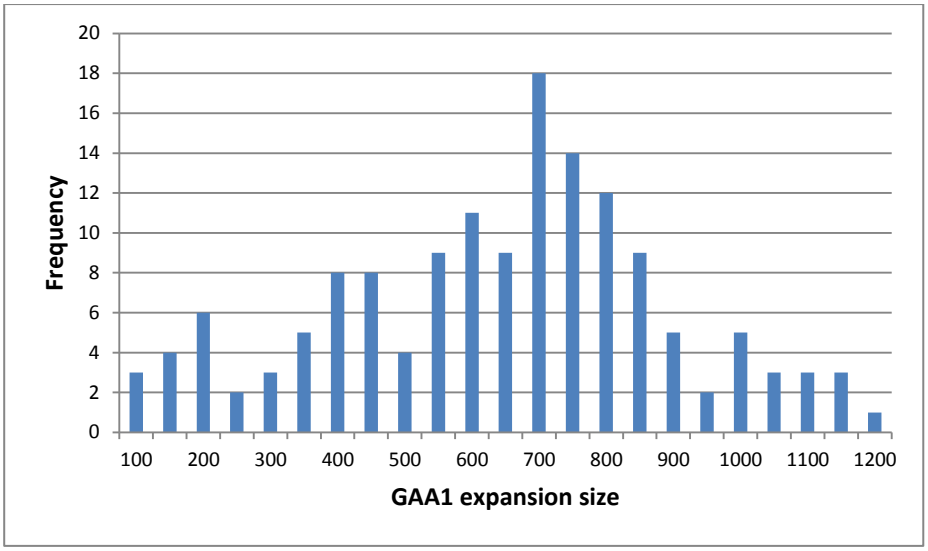
2.3.3 Genetic Features

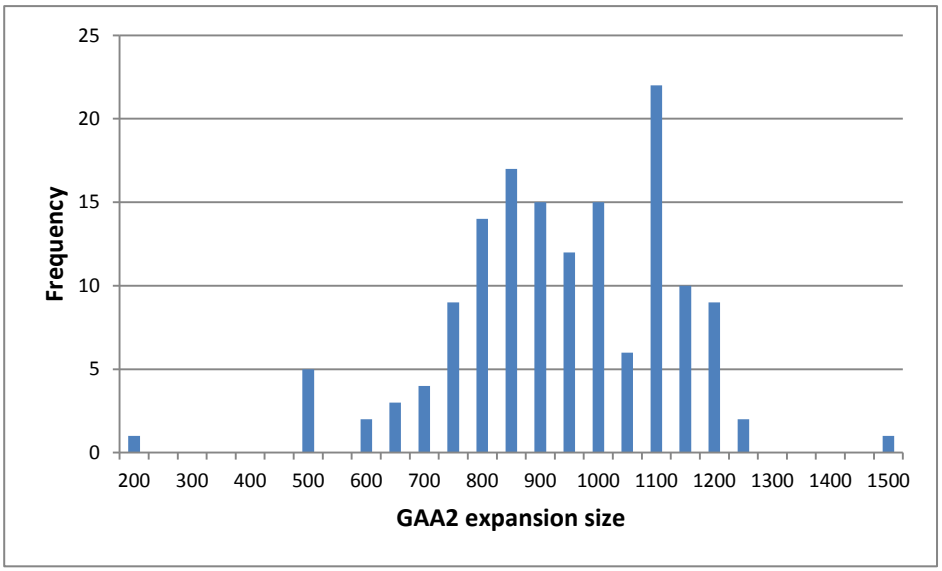
GAA expansion size results were available for 153 patients, principally from phase 1 of the study. This included six compound heterozygotes. Results for the remaining 14

patients were not available by the time of data analysis. Therefore, 147 results were available for patients with two GAA expansions. Analysis of the compound heterozygotes is presented in Chapter 3. By convention, the shorter of the two expansions is denoted by the term GAA1 and the longer, by GAA2. GAA1 sizes ranged from 100 to 1200 repeats with a mean value of 658.1 and mode of 700. GAA2 values varied from 200 to 1500 repeats with a mean value of 952.8 (see Table 4). The range of GAA expansion sizes is illustrated in Figure 10. There is a significantly longer tail of smaller GAA repeats amongst the GAA1 results below about 600 repeats when compared to the GAA2 results. This is perhaps because patients with two short expansions would have very mild symptoms and late onset or atypical presentations, and hence there is a negative ascertainment bias for these values. The distribution of GAA2 sizes is shifted approximately 200-400 repeats toward greater values, but is rather more skewed in the case of the GAA2 alleles. Thus, the maximum value for both alleles is roughly 1200 apart from a single outlier at 1500. It may be that values greater than this so disrupt the structure of the gene that they are not compatible with life.

Table 4: GAA expansion sizes for EFACTS patients (excluding compound heterozygotes)

	GAA1	GAA2
Mean	658.1	952.8
SD	254.2	186.2
Median	700	967
Range	100 - 1200	200 - 1500
n	147	147





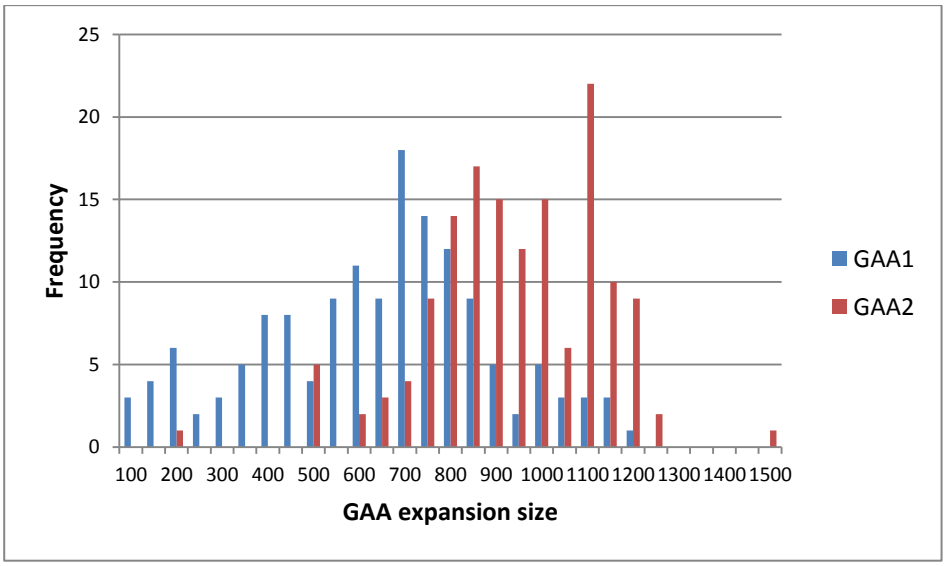


Figure 10: GAA expansion sizes for EFACTS patients with two GAA expansions
GAA1 (top graph) is defined as being the shorter of the two expansions, whereas GAA2 (middle graph) is the longer. The two sets of expansion sizes are merged in the bottom graph.

2.3.4 Onset & Disease Progression Recorded at Baseline

The mean age at onset of the FRDA patients in the study was 13.7, ranging from 1 to 55 years. 85.0% of the cohort used some aid for walking at least intermittently, from a mean age of 20. 74.9% of the cohort permanently used an aid for walking which occurred at a mean age of 22.1. Eighty-six of the patients in the study (51.5%) permanently used a wheelchair. Progression to permanent use of a wheelchair occurred at a mean age of 21.9 years. Further details of these milestones in disease progression are given in Table 5.

Table 5: Significant milestones in disease progression of EFACTS patients

		Age (years)
Age at onset	Mean ± SD	13.7 ± 9.6
(*n*=167)	Median	12
	Range	1-55
Age at intermittent support for walking	Mean ± SD	20.0 ± 10.5
(*n*=142)	Median	17
	Range	2-64
Age at permanent support for walking	Mean ± SD	22.1 ± 10.1
(*n*=125)	Median	19
	Range	2-65
Age at wheelchair-bound	Mean ± SD	21.9 ± 8.7
(*n*=86)	Median	19
	Range	5-48

The range of ages at symptom onset of the participating EFACTS patients is depicted in Figure 11 showing that the majority of patients have disease onset before the age of 20 but a small number have late-onset disease up to age 55. Figure 12 shows the range of ages at which patients become wheelchair-bound which most commonly occurs in teenage years or early twenties.

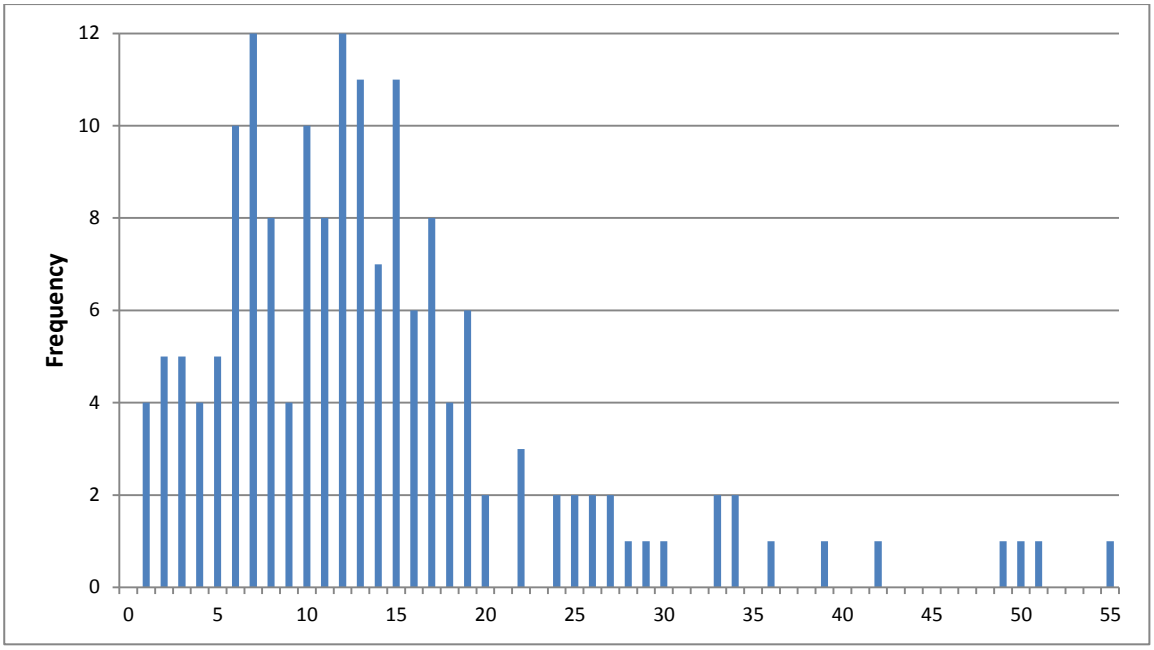


Figure 11: Age at onset of EFACTS patients
Age at onset varied from birth to age 55 but with the vast majority below the age of 25 as defined in Harding's diagnostic criteria

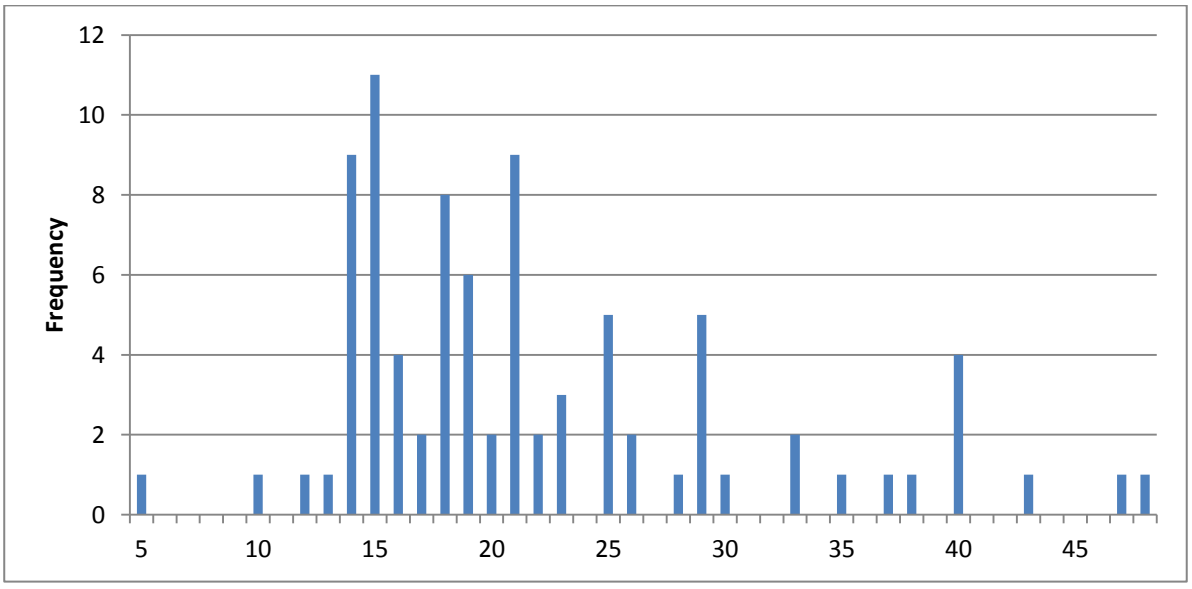


Figure 12: Age at wheelchair-bound for EFACTS patients
55% of the cohort required a wheelchair: the majority became wheelchair-bound between the ages of 15 and 25

The majority of patients present with instability, falls or clumsiness but a significant proportion (9.6%) first present with scoliosis. Initial presentation with other known non-neurological manifestations of FRDA such as skeletal foot abnormalities (2.4%), cardiomyopathy (1.2%) or diabetes (1.2%) is rare. A number of other rare presentations are recorded including pain (3%), fatigue (2.4%), dysarthria (1.2%),

postural dizziness (1.2%), stiffness (1.2%), urinary urge (0.6%), poor growth (0.6%) and cold intolerance (0.6%). Patients described several practical situations in which functional ability was first compromised such as problems with sport, carrying liquids or handwriting, which probably represent a combination of gait instability and loss of manual dexterity. Note, in this analysis, patients may present with more than one initial symptom, hence values add up to greater than 100%. The results are detailed in Table 6 and depicted in Figure 13.

Table 6: Symptoms at onset of EFACTS patients

Symptom at onset	Number (%)
Instability	112 (67.1)
Falls	26 (15.6)
Clumsiness	21 (12.6)
Scoliosis	16 (9.6)
Difficulty with sport	8 (4.8)
Difficulty carrying liquid	7 (4.2)
Altered gait	5 (3.0)
Leg/foot pain	5 (3.0)
Skeletal foot abnormality	4 (2.4)
Problem with handwriting	4 (2.4)
Fatigue	4 (2.4)
Cardiomyopathy	2 (1.2)
Dysarthria	2 (1.2)
Stiff limbs	2 (1.2)
Postural dizziness	2 (1.2)
Diabetes mellitus	1 (0.6)
Urinary urgency	1 (0.6)
Poor growth	1 (0.6)
Arrhythmia	1 (0.6)
Extreme reaction to alcohol	1 (0.6)
Episodic sensory symptoms	1 (0.6)
Cold intolerance	1 (0.6)
Not known	1 (0.6)

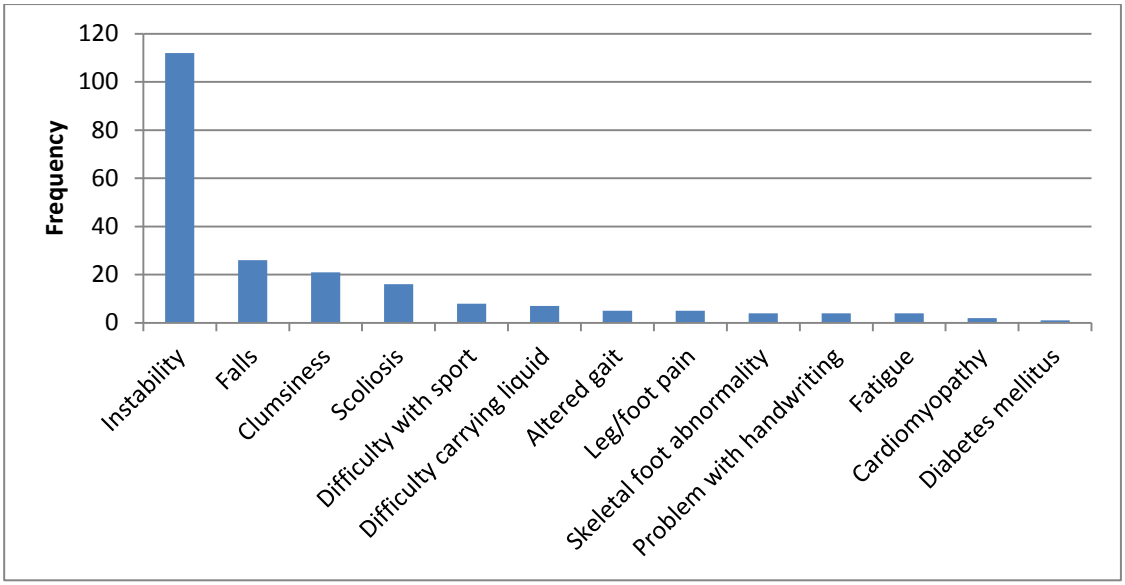


Figure 13: Common symptoms at onset of EFACTS patients
Instability is by far the commonest presenting feature followed by falls & clumsiness. Scoliosis is the only common non-neurological presenting feature

The level of disability of the EFACTS patients at baseline examination based on mobility was classified using the Spinocerebellar Degeneration Functional Scale (SDFS) which is a 7-point scale described in the Method above. The descriptions of the stages and proportion of patients with each stage are given in Table 7 and illustrated in Figure 14. 129 patients in the study complained of falls (77.2%) which started at a mean age of 17.1 ± 1.2 years (range 1 to 64 years). These figures show the heavy disease burden of FRDA at an early age.

Table 7: Spinocerebellar Degeneration Functional Score (SDFS) for EFACTS patients at baseline

Disability Stage	Number (%)
1: No functional handicap but signs at examination	1 (0.6)
2: Mild, able to run, walking unlimited	8 (4.8)
3: Moderate, unable to run, limited walking without aid	26 (15.6)
4: Severe, walking with one stick	14 (8.4)
5: Walking with two sticks	26 (15.6)
6: Unable to walk, requiring wheelchair	92 (55.1)
7: Confined to bed	0 (0)

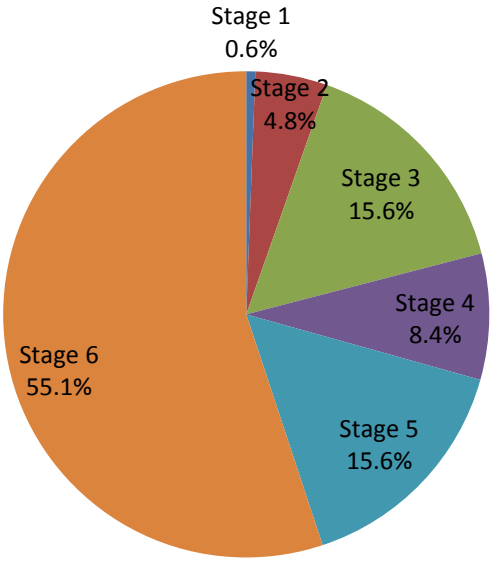


Figure 14: Spinocerebellar Degeneration Functional Score (SDFS) for EFACTS patients at baseline

2.3.5 Associated Clinical Features

Associated clinical features of FRDA were recorded in various parts of the EFACTS assessment at baseline. Visual and hearing problems, scoliosis, pes cavus and diabetes mellitus were recorded within the history section of the EFACTS Registry. Cardiac problems were recorded in this section and as part of a separate cardiology section. Further details were also recorded as part of the SNE which will be discussed later. Table 8 summarizes the medical problems recorded in the Registry for all the 167 EFACTS patients at baseline.

Table 8: General medical features seen in EFACTS patients at baseline

System	Diagnosis	Number (%)
Metabolic	Dysthyroid	7 (4.2)
	Hypercholesterolaemia	2 (1.2)
Cardiovascular	Cardiomyopathy/ventricular hypertrophy	72 (43.1)
	Hypertension	14 (8.4)
	Heart murmur	9 (5.4)
	Atrial fibrillation	8 (4.8)
	Supraventricular tachycardia	4 (2.4)
	Other arrhythmia/tachycardia	2 (1.2)
	Ischaemic heart disease/myocardial infarction	2 (1.2)
	Viral cardiomyositis	1 (0.6)
	Intracardiac thrombus	1 (0.6)

Respiratory	Asthma	18 (10.8)
	Obstructive sleep apnoea	5 (3.0)
	Pulmonary embolus	1 (0.6)
Gastrointestinal	Gastritis/GORD/Hiatus hernia	24 (14.4)
	Chronic constipation	11 (6.6)
	Chronic diarrhoea	2 (1.2)
	Coeliac disease	2 (1.2)
	Irritable bowel syndrome	2 (1.2)
	Haemorrhoids	2 (1.2)
	Pyloric stenosis	2 (1.2)
	Abdominal hernia	1 (1.2)
Urological	Urinary frequency/urge/incontinence/retention	36 (21.6)
	Suprapubic catheter	3 (1.8)
	Frequent urinary tract infections	3 (1.8)
	Haematuria	1 (0.6)
	Prostatic enlargement	1 (0.6)
	Erectile dysfunction	1 (0.6)
	Hypospadias	1 (0.6)
	Nephrolithiasis	1 (0.6)
Gynaecological	Dysmenorrhoea	3 (1.8)
	Hysterectomy/ovariectomy	1 (0.6)
	Ovarian malignancy	1 (0.6)
Ophthalmological	Myopia	48 (28.7)
	Hypermetropia	18 (10.8)
	Nystagmus/oscillopsia	15 (9.0)
	Optic neuropathy	9 (5.4)
	Strabismus (operated)	3 (1.8)
	Astigmatism	3 (1.8)
	Keratoconus	2 (1.2)
	Amblyopia	1 (0.6)
	Presbyopia	1 (0.6)
	Corneal ulceration	1 (0.6)
	Epiretinal membrane	1 (0.6)
	Glaucoma	1 (0.6)
	Diabetic retinopathy	1 (0.6)
	Mears-Irlen syndrome	1 (0.6)
Otorhinolaryngological	Tonsillectomy/adenoidectomy	4 (2.4)
	Grommets/multiple ear infections	2 (1.2)
	Mastoid perforation/abscesses/ear infections	1 (0.6)
	Small external meati/dewaxing/ear infections	1 (0.6)
	Chronic otitis externa	1 (0.6)
	Skull fracture with hearing loss	1 (0.6)
	Rhinoplasty with sinusitis	1 (0.6)
	Prominent epistaxis	1 (0.6)
	Bruxism	1 (0.6)
	Post-nasal drip	1 (0.6)

Rheumatological	Osteoporosis	4 (2.4)
	Arthritis	2 (1.2)
Orthopaedic	Bony fracture	14 (8.4)
	Meniscectomy	3 (1.8)
	Hip replacement	2 (1.2)
	Joint dislocation	1 (0.6)
	Avascular necrosis of the scaphoid	1 (0.6)
Dermatological	Eczema	12 (7.2)
	Chronic pruritis	3 (1.8)
	Psoriasis	2 (1.2)
	Acne	1 (0.6)
	Shingles	1 (0.6)
	Cutaneous sarcoidosis	1 (0.6)
Haematological	Iron deficiency anaemia	7 (4.2)
	B12/folate deficiency	4 (2.4)
	Haemolytic anaemia (glandular fever)	1 (0.6)
	Anaemia, cause unknown	2 (1.2)
	Deep vein thrombosis	2 (1.2)
	Superficial thrombophlebitis	1 (0.6)
	Thombophilia/Factor V Leiden	2 (1.2)
	Beta-thalassaemia	1 (0.6)
Psychiatric	Depression	15 (9.0)
	Anxiety/panic attacks	11 (6.6)
	Adjustment disorder	1 (0.6)
	Tourette's syndrome	1 (0.6)
Neurological	Migraine	8 4.8)
	Epilepsy	6 (3.6)
	Multiple sclerosis	2 (1.2)
	Parkinson's disease	1 (0.6)
	Subarachnoid haemorrhage	1 (0.6)

GORD=Gastro-oesophageal reflux disease
Cardiac, ophthalmological & otorhinolaryngological diagnoses are discussed in the text

The measures of visual impairment and hearing loss recorded in the EFACTS Registry do not discriminate between visual and hearing loss primarily caused by FRDA, and those of different origin. Given that the majority of participants are young, most hearing loss recorded is likely to be a direct result of FRDA. General medical problems are recorded as part of the EFACTS assessment. The ear, nose and throat section documents only six alternative causes of hearing loss, *viz*. grommets and/or multiple ear infections (2 cases); mastoid perforation and abscesses (1 case); small external auditory meati/frequent dewaxing/multiple ear infections (1 case); chronic otitis externa (1 case); and hearing loss caused by skull fracture (1 case). The number of

cases directly attributable to be FRDA is therefore likely to be around 54 or 32.3% of the total cohort. Twenty-three patients (13.8%) are recorded as currently or previously using a hearing aid (including four of the above six patients). Only three patients felt that their hearing aid was effective (all cases attributable to FRDA). One patient had a cochlear implant inserted as a child.

A high proportion of patients are recorded in the EFACTS Registry as having visual impairment (116 cases or 69.5% of the total cohort). However, this figure includes all cases of visual impairment including such common ophthalmic problems as myopia (48 cases), hypermetropia (18 cases), presbyopia (1 case) and astigmatism (3 cases) which are unlikely to be directly caused by FRDA. Ninety-six patients (57.5%) are recorded in the Registry as using spectacles; in 75 cases these are recorded as being effective. The Registry formally records nine patients as having optic neuropathy and fifteen patients as having visual problems related to nystagmus, oscillopsia or abnormalities of visual fixation. There are also eleven patients with other causes of visual impairment (3 cases of strabismus, 2 cases of keratoconus and 1 case each of corneal ulceration, epiretinal membrane, amblyopia, Mears-Irlen syndrome, glaucoma and diabetic retinopathy). None of the above categories is mutually exclusive and so that patients may have multiple causes of visual impairment. Visual impairment in FRDA is caused by degeneration of both the anterior (optic nerve) and posterior (optic radiation and visual cortex) visual pathways, as well as oculomotor problems (Fortuna *et al.* 2009, Noval *et al.* 2012). There are 24 cases (14.4%) in the EFACTS Registry in which the cause of visual impairment is formally attributed to one of these or not recorded as having an alternative cause which is unrelated to FRDA. This figure is therefore likely to represent the prevalence of visual impairment in FRDA which is directly attributable to the condition itself.

116 patients in the Registry have scoliosis (69.5%), of which 75 are recorded as mild, 25 moderate, 10 severe and 6 of unknown severity. Thirty-eight have undergone corrective surgery at a mean age of 15.2 (range 6-21). Ninety-seven patients have pes cavus (58.1%), of which 30 are recorded as mild, 42 moderate, 20 severe and 5 of unknown severity. Twelve patients have undergone corrective surgery on their feet at a mean age of 23.8 (range 10-40).

Fifteen patients are recorded in the Registry as having diabetes mellitus, including ten with type 1 diabetes, four with type 2 and one of unknown type. The mean age at onset of diabetes mellitus was 25.5 (range 4-53).

Dysuria was seen in 36 patients (21.6%) and frequent urinary tract infections in three (1.8%). Gastritic symptoms or hiatus hernia were described in 24 patients (14.4%) whilst chronic constipation was seen in 11 (6.6%). Fourteen patients had sustained a bony fracture (8.4%). Fifteen patients (9.0%) gave a history of depression and 11 (6.6%) a history of anxiety or panic attacks. Asthma was seen in 18 patients (10.8%). The common associated clinical features of FRDA are shown in Table 9 and depicted in the Discussion in Figure 36.

Table 9: Frequency of associated clinical features for EFACTS patients at baseline

	Number (%)
Visual impairment	116 (69.5)
-attributable to FRDA[a]	24 (14.4)
Hearing loss	60 (35.9)
-attributable to FRDA[a]	54 (32.3)
Scoliosis	116 (69.5)
Pes cavus	97 (58.1)
Cardiomyopathy	72 (43.1)
Diabetes mellitus	15 (9.0)
Urinary dysfunction	36 (21.6)
Gastritic symptoms	24 (14.4)

[a]see explanation in text

Seventy-two of the participants in the study (43.1%) had a history of cardiomyopathy. Twenty-six (15.6%) had a history of arrhythmia of which eight were known to have atrial fibrillation and four supraventricular tachycardia. The precise diagnosis was unclear in the remainder. Three patients (1.8%) had a permanent cardiac pacemaker *in situ.* Fourteen patients (8.4%) suffered with hypertension. Nine patients (5.4%) had a history of a heart murmur either currently or in the past (including childhood). Two patients (1.2%) had a diagnosis of ischaemic heart disease. One patient had previously had an intracardiac thrombus diagnosed during pregnancy. One patient previously had viral cardiomyositis.

Table 10 shows the results of cardiac investigations of the EFACTS patients at baseline.

In total, echocardiographic data were available for 104 patients, and ECG data for 91. Results are recorded as a percentage of values obtained rather than of the whole cohort which may overestimate the prevalence of abnormal findings as patients with evidence of cardiac involvement are more likely to be referred for cardiac assessment. The seven cases of conduction abnormality included three cases of incomplete right bundle branch block, two cases of incomplete left bundle branch block, one case of long QT interval and one case of a non-specific intraventricular conduction delay.

Table 10: Results of cardiac investigations at baseline for EFACTS patients

Investigation		Value (%)	*n*
Systolic BP (mmHg)	Mean ± SD	116.7 ± 17.9	164
	Range	66 – 182	
Diastolic BP (mmHg)	Mean ± SD	78.6 ± 14.5	164
	Range	45 – 168	
Pulse (bpm)	Mean ± SD	77.9 ± 12.3	164
	Range	52 - 112	
IVSd (mm)	Mean ± SD	10.6 ± 2.6	91
	Range	6 - 20	
LVPWd (mm)	Mean ± SD	10.0 ± 2.6	86
	Range	6 - 17	
LVEF (%)	Mean ± SD	63.2 ± 10.9	87
	Range	29 - 89	
ECG	Repolarization changes	59 (69.4%)	88
	Voltage criteria for LVH	28 (40.0%)	70
	Pathological Q waves	7 (11.1%)	63
	Conduction abnormalities	7 (10.6%)	66
	Arrhythmia (all AF)	3 (3.4%)	89
	Paced rhythm	3 (3.3%)	90

Reference values[a]	Normal	Mild	Moderate	Severe
IVSd (mm)	6 – 12	13 - 15	16 - 19	≥20
LVPWd (mm)	6 – 12	13 - 15	16 - 19	≥20
LVEF (%)	≥55%	45-54%	36-44%	≤35%

IVSd=intraventricular septal thickness at diastole
LVPWd=left ventricular posterior wall thickness at diastole
LVEF=left ventricular ejection fraction
ECG=electrocardiogram BP=Blood pressure
LVH=left ventricular hypertrophy AF=atrial fibrillation
[a] from (Steeds *et al.* 2011)

2.3.6 Activities of Daily Living (ADL)

164 participants completed the ADL questionnaire at baseline, as described in the Method at 2.2.2. The individual questions of the ADL questionnaire can be found in the Appendix. Figure 15. The mean value was 15.6 ± 8.4 out of a possible total of 36. The walking and falls parameters were most severely affected, with swallowing and bladder function least affected. Higher values represent greater disability.

Table 11 shows the results by subscore of the questionnaire which are shown graphically in Figure 15. The mean value was 15.6 ± 8.4 out of a possible total of 36. The walking and falls parameters were most severely affected, with swallowing and bladder function least affected. Higher values represent greater disability.

Table 11: Subscores of the Activities of Daily Living section of the FRDA Rating Scale for EFACTS patients at baseline

Score	Speech	Swallow	Use of Cutlery	Dressing	Washing	Falls	Walking	Sitting	Bladder Function	Total
0	18	45	41	35	42	29	1	42	96	349
1	52	60	48	44	48	41	19	33	37	382
2	76	55	34	34	29	16	13	37	14	308
3	13	2	27	32	27	8	43	34	9	195
4	5	2	14	19	18	70	88	18	8	242
Mean	1.6	1.1	1.5	1.7	1.6	2.3	3.2	1.7	0.8	15.6
SD	0.9	0.9	1.3	1.3	1.3	1.6	1.0	1.3	1.1	8.4
Mode	2	1	1	1	1	4	4	0	0	1

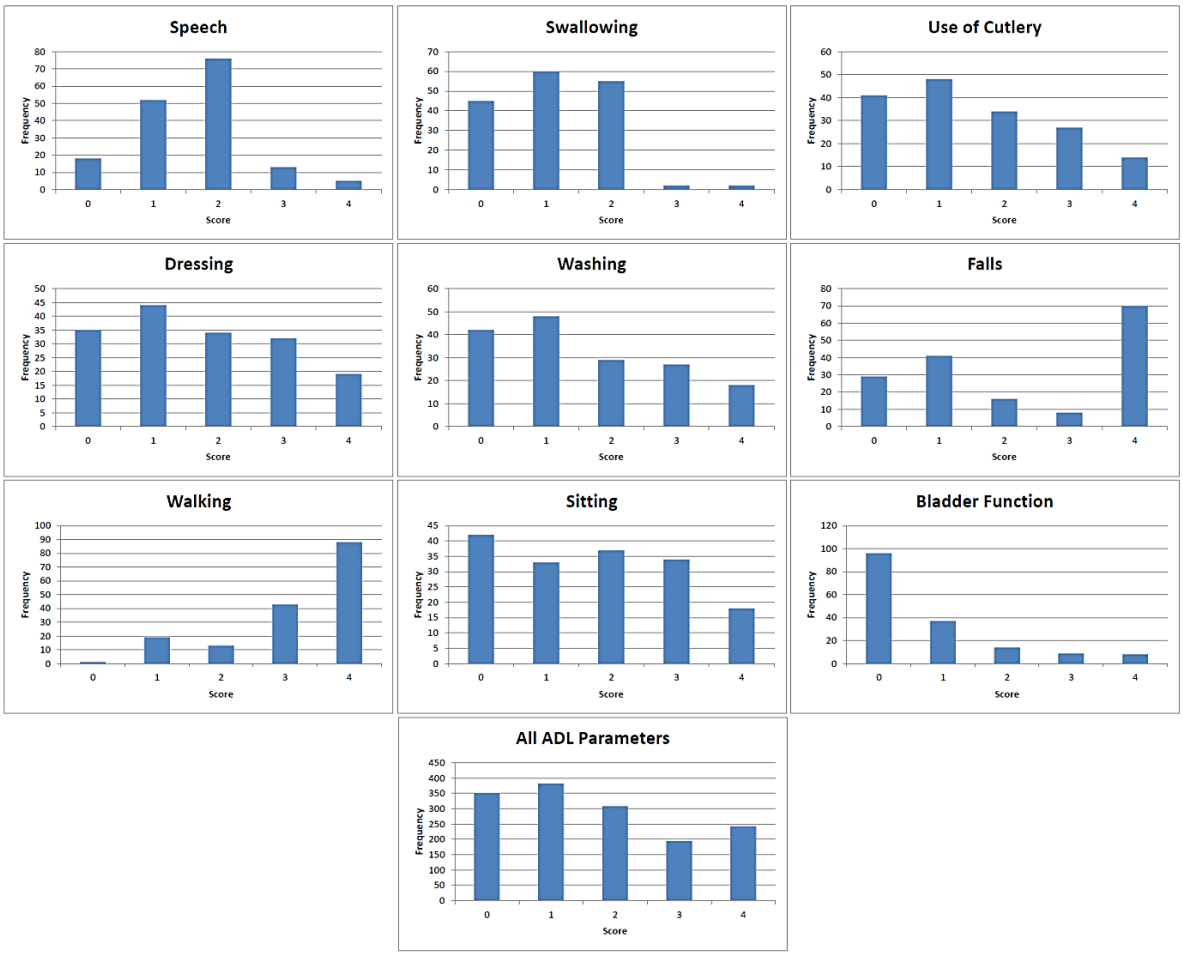


Figure 15: Subscores of the Activities of Daily Living section of the FRDA rating scale for EFACTS patients at baseline

Most subscores show a broad range of values except falls and walking which are skewed to higher values, and bladder function which is skewed to lower values

2.3.7 Scale for the Assessment & rating of Ataxia (SARA)

The SARA was assessed on 166 FRDA patients at baseline, as described in the Method at 2.2.2. The individual subscores can be found in the Appendix. The results are shown in Table 12 and depicted graphically in Figure 16. The mean SARA was 22.5±10.0. The gait, stance, sitting and heel-shin test subscores had the greatest proportion in the highest scores, indicating severe involvement of these parameters. The speech, finger chase, finger-nose test and fast alternating hand movement results were more evenly spread, indicating less severe involvement of these functions. Fractional values appear for the finger chase, finger-nose test, fast alternating hand movements and heel-shin test subscores as these parameters are assessed for each limb independently and a

mean calculated. Fractional values are much less common than integral values as patients often have symmetrical signs. Higher values represent more prominent ataxia.

Table 12: SARA subscores for FRDA patients at baseline

Score	Gait	Stance	Sitting	Speech	Finger Chase	Nose-Finger Test	Fast Alternating Hand Movements	Heel-Shin Test
0	0	4	44	29	15	16	24	5
0.5	-	-	-	-	7	10	11	9
1	10	17	31	33	73	46	46	25
1.5	-	-	-	-	4	14	12	4
2	16	20	12	57	46	33	15	26
2.5	-	-	-	-	1	4	6	0
3	8	11	1	33	11	36	43	25
3.5	-	-	-	-	0	0	0	1
4	3	3	78	10	9	7	9	71
5	10	16	-	1	-	-	-	-
6	16	95	-	3	-	-	-	-
7	7	-	-	-	-	-	-	-
8	96	-	-	-	-	-	-	-
Mean	6.3	4.5	2.2	1.9	1.5	1.7	1.7	2.7
SD	2.4	2.0	1.8	1.3	1.0	1.1	1.2	1.3
Mode	8	6	4	2	1	1	1	4

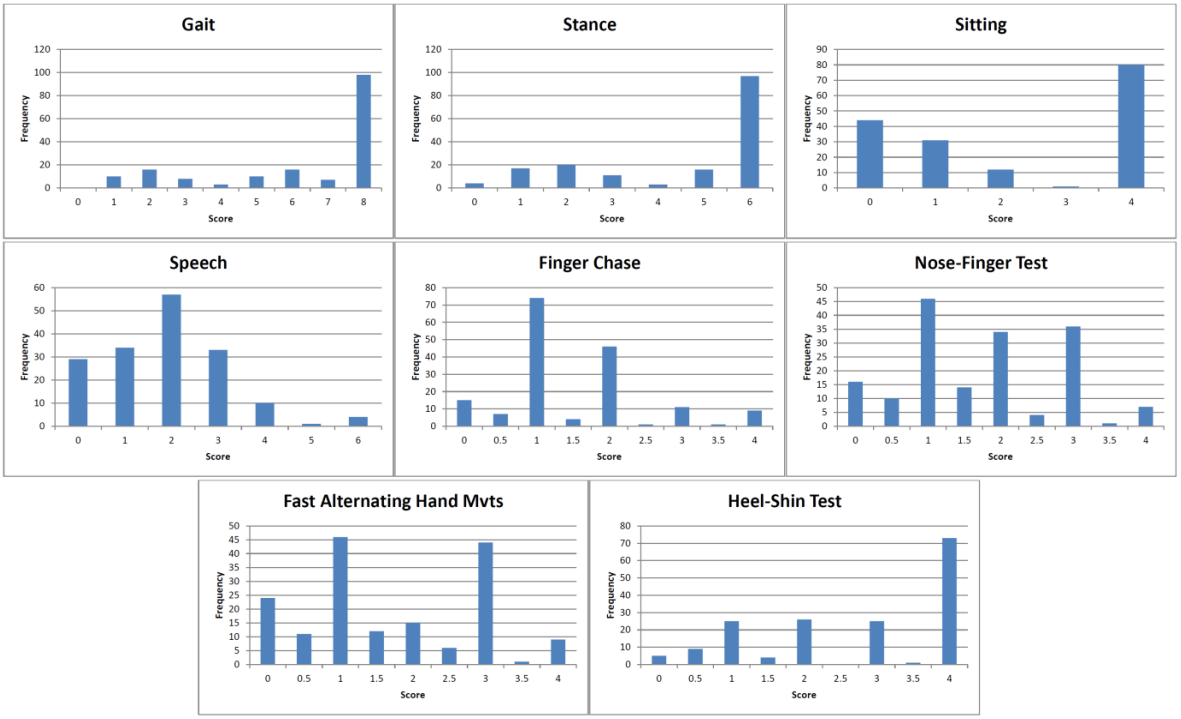


Figure 16: SARA subscores for FRDA patients at baseline

All the subscores involved in LL function are skewed toward higher values. Those for UL function and speech are more broadly spread

2.3.8 Inventory of Non-Ataxic Symptoms (INAS)

The INAS was completed at baseline for 166 patients in the study, as described in the Method at 2.2.2. The individual features of the INAS can be found in the Appendix. Higher values represent greater disease involvement. The mean INAS count was 5.0 ± 1.6. The number and proportion of participants having each of the INAS count values are displayed in Table 13 and illustrated in Figure 17. Areflexia, sensory loss and weakness are seen in more than 75% of cases. Extensor plantar reactions, spasticity, amyotrophy, urinary dysfunction and brainstem oculomotor signs are seen in between a quarter and three quarters of patients. Hyperrefexia and cognitive dysfunction are seen in less than 10% of cases, whilst dystonia, myoclonus, chorea and parkinsonism were seen in less than 3%. Indeed, the only cases of resting tremor and rigidity were observed in a 50-year old patient who was compound heterozygous for a p.Gly130Val mutation and who was felt to have an independent diagnosis of Parkinson's disease (see Chapter 3, Patient 2 for further details).

Table 13: INAS count values for EFACTS patients at baseline

	No. of patients (%)
Hyperreflexia	11 (6.6)
Areflexia	156 (94.0)
Extensor plantar	104 (62.7)
Spasticity	80 (48.2)
Paresis	129 (77.7)
Muscle atrophy	76 (45.8)
Fasciculations	1 (0.6)
Myoclonus	2 (1.2)
Rigidity	1 (0.6)
Chorea/dyskinesia	3 (1.8)
Dystonia	4 (2.4)
Resting tremor	1 (0.6)
Sensory symptoms	146 (88.0)
Urinary dysfunction	63 (38.0)
Cognitive dysfunction	13 (7.8)
Brainstem oculomotor signs	47 (28.3)

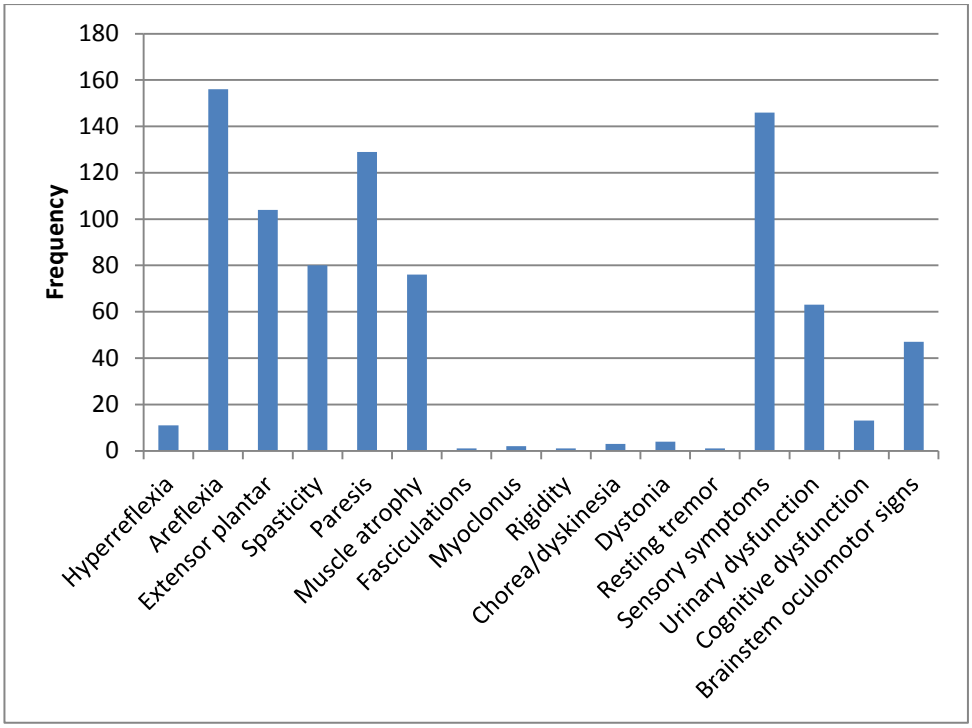


Figure 17: INAS count values for EFACTS patients at baseline

Table 14 details the pattern of non-ataxic signs and symptoms as recorded in raw data from the INAS for the EFACTS cohort at the baseline assessment. The pattern of weakness, spasticity and vibrational sensory loss is illustrated in Figure 18; symptoms reported by the patients as part of the INAS (diplopia, dysphagia, episodic vertigo, dysarthria, handwriting problems and muscular cramps) are shown in Figure 19; the results of deep tendon reflexes are shown in Figure 20; finally, the ophthalmological features of the EFACTS cohort at baseline are represented in Figure 21.

Table 14: Non-ataxic symptoms & signs from INAS at baseline for EFACTS patients [Number (%)]

		None	Mild	Moderate	Severe
Weakness	UL proximal	131 (78.9)	27 (16.3)	3 (1.8)	5 (3.0)
	UL distal	98 (59.0)	41 (24.7)	14 (8.4)	13 (7.8)
	LL proximal[a]	51 (30.7)	45 (27.1)	34 (20.5)	34 (20.5)
	LL distal[b]	48 (28.9)	33 (18.1)	24 (14.5)	59 (35.5)
Spasticity	UL[c]	121 (72.9)	41 (24.7)	3 (1.8)	0 (0)
	LL[d]	99 (59.6)	33 (19.9)	24 (14.4)	6 (3.6)
Impaired vibration	LL distal[e]	24 (14.5)	38.5 (23.2)	46.5 (28.0)	54 (32.5)

	None	Mild	Moderate	Severe
Diplopia	143 (86.1)	17 (10.2)	3 (1.8)	3 (1.8)
Dysphagia	52 (31.3)	64 (38.6)	46 (27.7)	4 (2.4)
Episodic vertigo	142 (85.5)	9 (5.4)	9 (5.4)	6 (3.6)

Speech problems	18 (10.8)	64 (38.6)	62 (37.3)	22 (13.3)
Handwriting problems	18 (10.8)	24 (14.5)	72 (43.4)	52 (31.3)
Muscle cramps	58 (34.9)	37 (22.3)	17 (10.2)	54 (32.5)

	Normal	**Hyperreflexic**	**Areflexic**
Biceps reflex	10 (6.0)	6 (3.6)	150 (90.4)
Patellar reflex	15 (9.0)	10 (6.0)	141 (84.9)
Achilles reflex	13 (7.8)	3 (1.8)	150 (90.4)
	None	**Unilateral**	**Bilateral**
Extensor Plantar[f]	62 (37.3)	10 (6.0)	92 (55.4)

	Number (%)
Broken smooth pursuits	131 (78.9)
Square wave jerks on fixation	108 (65.0)
Downbeat nystagmus on fixation	1 (0.6)
Gaze-evoked nystagmus on horizontal testing	61 (36.7)
Gaze-evoked nystagmus on vertical testing	14 (8.4)
Ophthalmoparesis on horizontal gaze[g]	4 (2.4)
Ophthalmoparesis on vertical gaze[h]	8 (4.8)
Slowing of saccades	44 (26.5)
Hypometric saccades[i]	94 (56.6)
Hypermetric saccades[j]	82 (49.4)

UL=upper limb ; LL=lower limb

Missing values: [a]*n*=2 ; [b]*n*=2; [c]*n*=1; [d]*n*=4; [e]*n*=4; [f]*n*=2; [g]*n*=1; [h]*n*=1; [i]*n*=5; [j]*n*=4

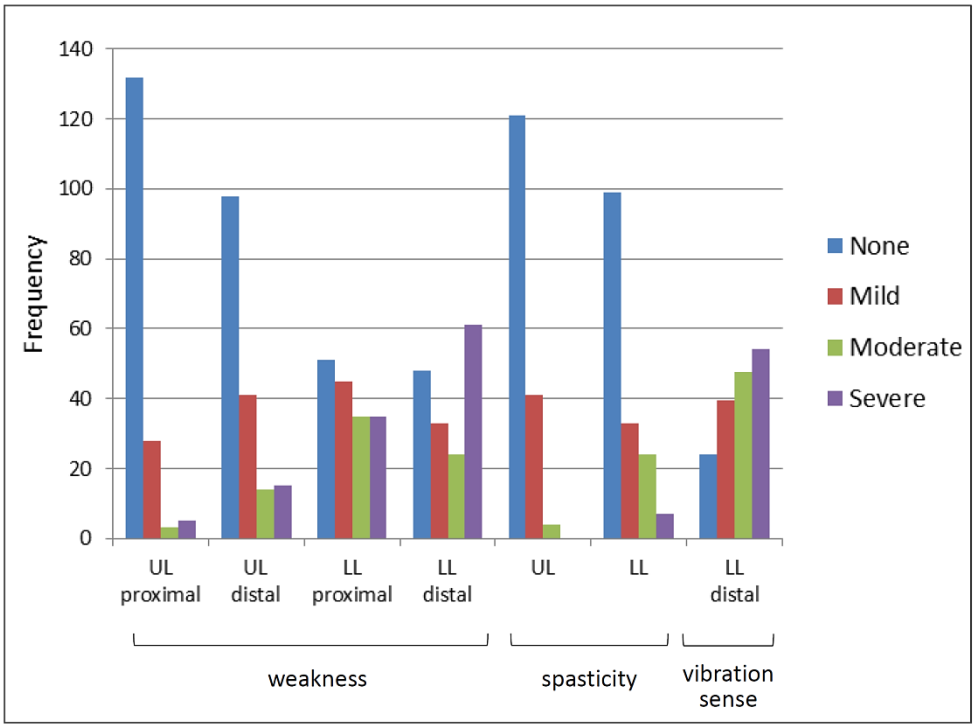


Figure 18: Weakness, spasticity & sensory loss for EFACTS patients as recorded in the INAS at baseline

LL weakness is much commoner than UL. Spasticity is rare but commoner in the LLs. LL distal sensory loss is common

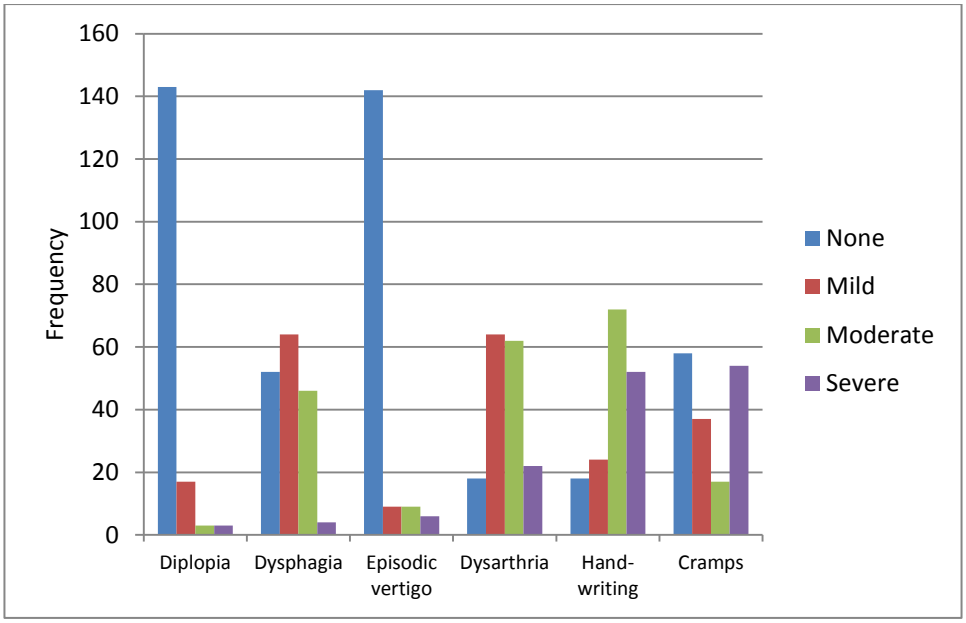


Figure 19: Symptoms reported by EFACTS patients at baseline as part of the INAS
Dysphagia, dysarthria, problems with handwriting and muscular cramps are much commoner than diplopia and vertigo in FRDA

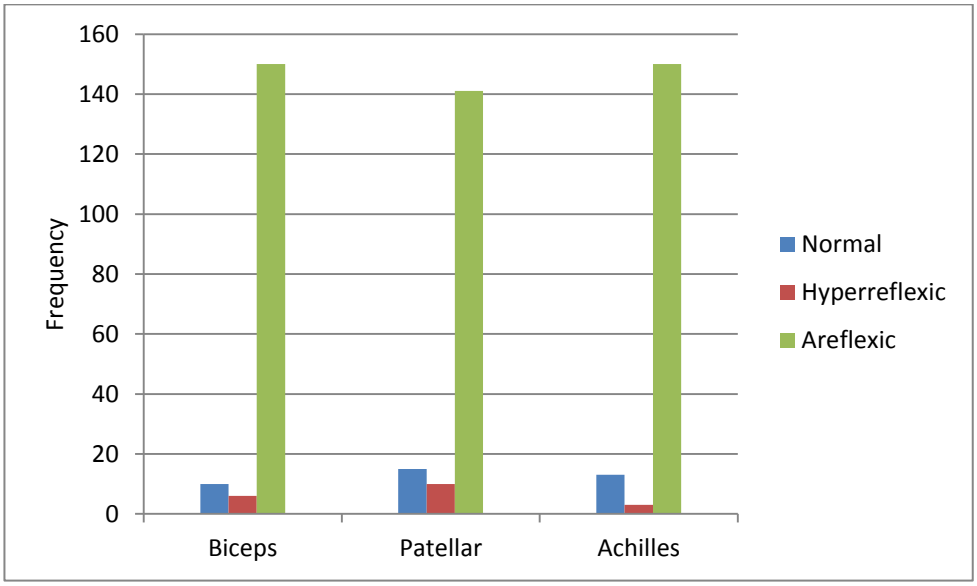


Figure 20: Reflexes for EFACTS patients as recorded in the INAS at baseline
Areflexia is common throughout in FRDA

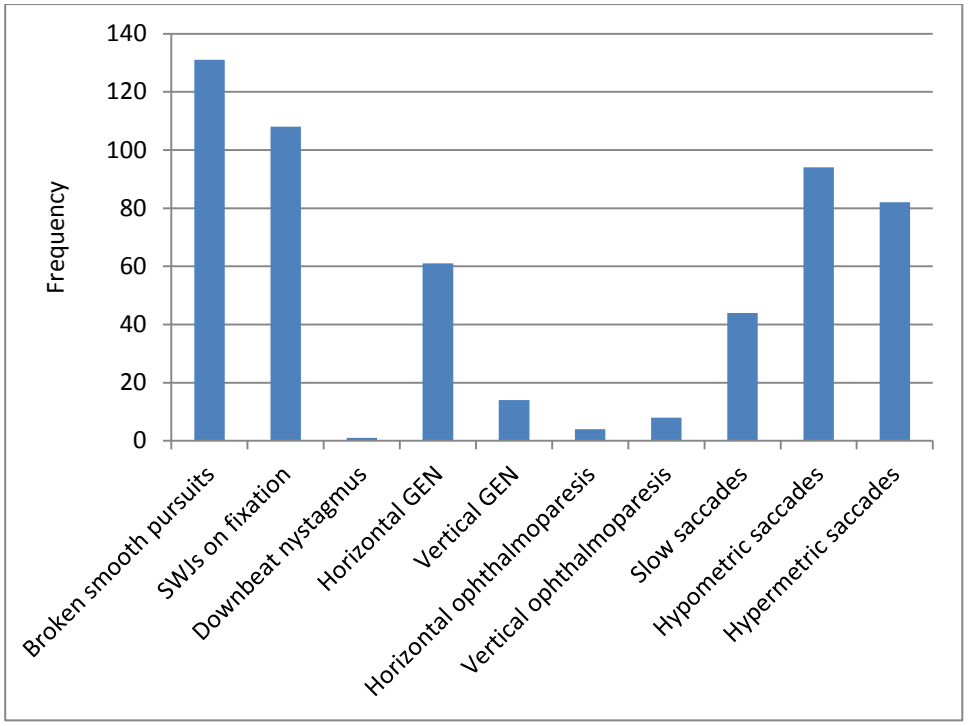


Figure 21: Ophthalmological features of EFACTS patients as recorded in the INAS at baseline

2.3.9 Spinocerebellar Ataxia Functional Index (SCAFI)

SCAFI data were available at baseline for 155 patients from the EFACTS cohort, as described in the Method at 2.2.2. The individual features of the SCAFI can be found in the Appendix. All 155 (92.8%) completed the speech assessment (PATA test); 110 patients (65.9%) completed the 9-hole peg test (9HPT) with both hands; however, just 53 patients (31.7%) completed the 8-metre timed walk (8mTW). Lower values for the PATA test and higher values for the 8mTW and 9HPT represent greater disease involvement. 138 are recorded as being right-handed (82.6%); 14 are recorded as left-handed (8.4%); handedness was not recorded in 12 cases when it was clear that the patient was unable to perform the 9HPT. Of the 12 patients for whom PATA test data are unavailable, only three were recorded in the speech component of the ADLs as being 'mostly incomprehensible' suggesting that the reason the test was not performed was not always because of anarthria. Other factors may have included fatigue, refusal on the part of the patient or lack of clinic time.

It is known from the SDFS that 55.1% of the cohort is wheelchair-bound and so unable to perform the 8mTW. In addition, many patients whilst remaining mobile at home

with assistive devices, attend clinic in a wheelchair and do not bring potentially bulky mobility aids with them, and so may have been unable to complete this task on the day. At the baseline assessment, five patients used a wheeled walker, six patients used two sticks, eleven patients used one stick and 29 patients did not use a walking aid. No information was recorded for three patients.

Similarly, lack of manual dexterity is a common reason for being unable to complete the 9HPT. This test has a cut-off value of 5 minutes above which patients are deemed unable to complete the task. It is not uncommon for patients to reach this limit. Taken together, these observations demonstrate one of the major failings of the SCAFI as a rating scale in FRDA, as a high proportion of patients are physically incapable of completing all parts of the test.

The results of the raw data are summarized in Table 15 and shown graphically in Figure 22. The 8mTW & 9HPT data are skewed toward lower values, particularly the 8mTW values. Only the PATA tests data are normally distributed. The distribution of results for the 9HPT for the dominant and non-dominant hand is similar, but the values for the non-dominant hand are slightly greater – typically by about 10s – as would be expected.

Table 15: Raw data from SCAFI for EFACTS patients at baseline

Task	No. completing (%)	Mean ± SD	Range
8mTW (s)	53 (31.7)	15.0 ± 20.8	4 – 135
9HPT-D[1] (s)	120 (71.9)	69.1 ± 47.4	19 – 253
9HPT-ND[2] (s)	110 (65.9)	69.6 ± 41.8	21 – 244
9HPT-All[3] (s)	115 (68.9)	69.3 ± 44.7	19 – 253
PATA (/10s)	155 (92.8)	15.3 ± 4.9	1 - 33

[1]9HPT-D = 9-hole peg test for dominant hand
[2]9HPT-ND = 9-hole peg test for non-dominant hand
[3]9HPT-All = 9-hole peg test for both hands

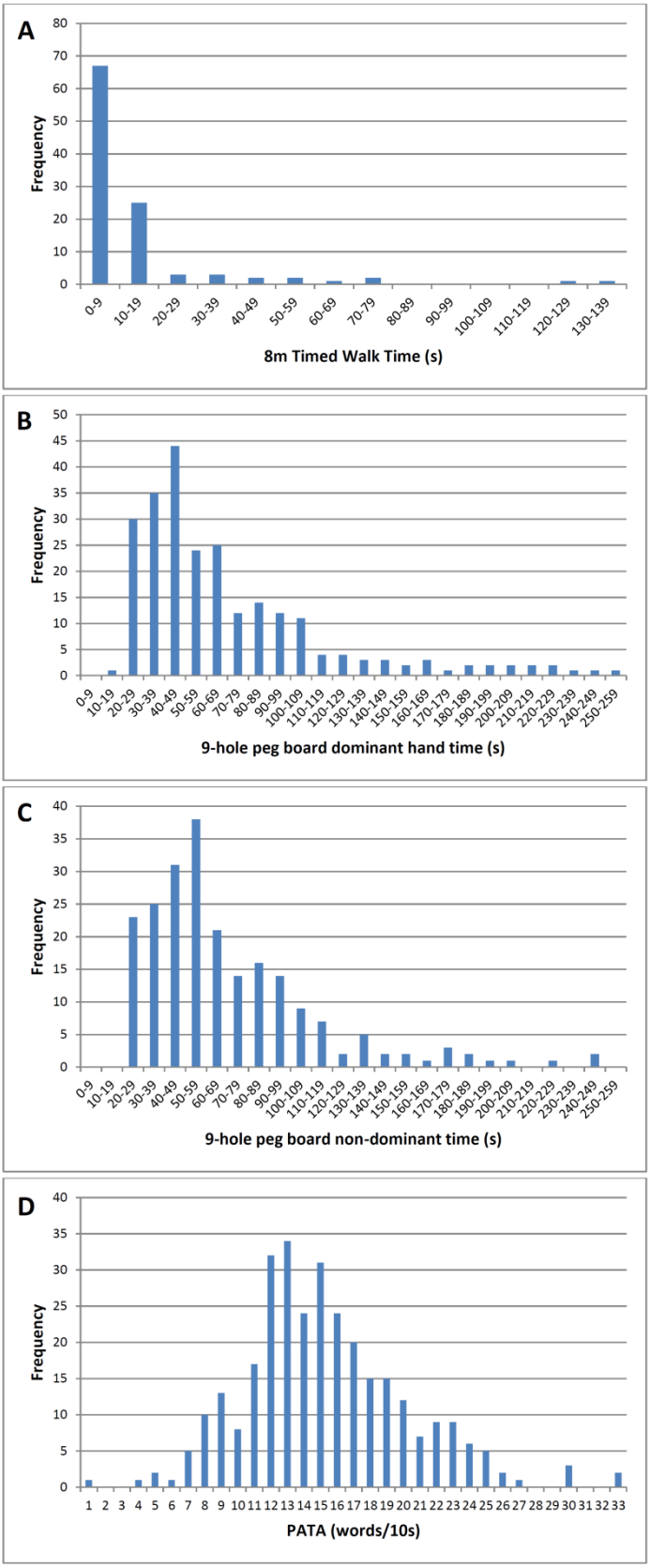


Figure 22: Raw data from SCAFI for EFACTS patients at baseline

(A) 8m timed walk; (B) 9-hole peg test time for dominant hand; (C) 9-hole peg test time for non-dominant hand; (D) PATA test

2.3.10 Structured Neurological Examination (SNE)

Data from the SNE were available for 158 EFACTS patients participating although there were numerous instances of missing data in individual fields, hence the number of patients examined for each field is recorded. Missing data usually arose as a result of lack of time in clinic. The SNE is described in detail in the Method at 2.2.2 and the SNE proforma can be found in the Appendix. Ptosis was seen in 25 out of 151 patients (16.6%). One patient out of 146 had a relative afferent pupillary defect (0.7%). There were no documented cases of facial sensory loss. Five out of 153 patients (3.3%) had facial weakness. There was one out of 152 (0.7%) cases of masticatory muscle weakness recorded. Four out of 148 patients (2.7%) had weakness of palatal elevation. One out of 152 patients (0.7%) had sternocleidomastoid weakness. Three out of 152 patients (2.0%) had trapezius weakness. There were seven out of 144 (4.9%) cases of tongue atrophy recorded. Out of 141 cases recorded, lingual tone was flaccid in four cases (2.8%), normal in 79 (56.0%), spastic in 47 (33.3%) and highly spastic in 11 (7.8%).

The range of muscle power found at examination is given in Table 16 with each value the result of two measurements (*ie* one each side) for all patients (*ie n* measurements in total). The scale is described in the Method at 2.2.2 above with 0 representing no power and 7, full power. The mean values are depicted in Figure 23.

Table 16: Muscle power from SNE for EFACTS patients

	Mean	SD	Mode	Range	*n*
Shoulder Abduction	6.46	1.15	7	0 - 7	316
Shoulder Adduction	6.65	0.99	7	0 - 7	316
Elbow Flexion	6.78	0.80	7	2 - 7	316
Elbow Extension	6.78	0.88	7	1 - 7	316
Wrist Flexion	6.56	1.17	7	0 - 7	316
Wrist Extension	6.66	0.98	7	0 - 7	316
Finger Flexion	6.55	1.08	7	0 - 7	312
Finger Extension	6.13	1.54	7	0 - 7	314
Index Finger Abduction	5.72	1.90	7	0 - 7	314
Little Finger Abduction	5.84	1.88	7	0 - 7	314
Thumb Abduction	5.82	1.91	7	0 - 7	314
UL proximal	6.67	0.97	7	0 - 7	632
UL distal	6.18	1.59	7	0 - 7	2200
Hip Flexion	4.24	2.38	7	0 - 7	312
Hip Extension	4.64	2.38	7	0 - 7	310
Hip Abduction	4.50	2.76	7	0 - 7	308

Hip Adduction	4.54	2.73	7	0 - 7	308
Knee Flexion	3.79	2.65	7	0 - 7	312
Knee Extension	5.54	2.17	7	0 - 7	312
Ankle Flexion	4.31	2.90	7	0 - 7	306
Ankle Extension	4.16	2.86	7	0 - 7	310
Ankle Inversion	3.78	3.07	7	0 - 7	302
Ankle Eversion	3.62	3.10	7	0 - 7	302
Toe Flexion	3.82	3.02	7	0 - 7	302
Toe Extension	3.86	2.99	7	0 - 7	306
LL proximal	4.54	2.57	7	0 - 7	1582
LL distal	3.93	3.00	7	0 - 7	1828

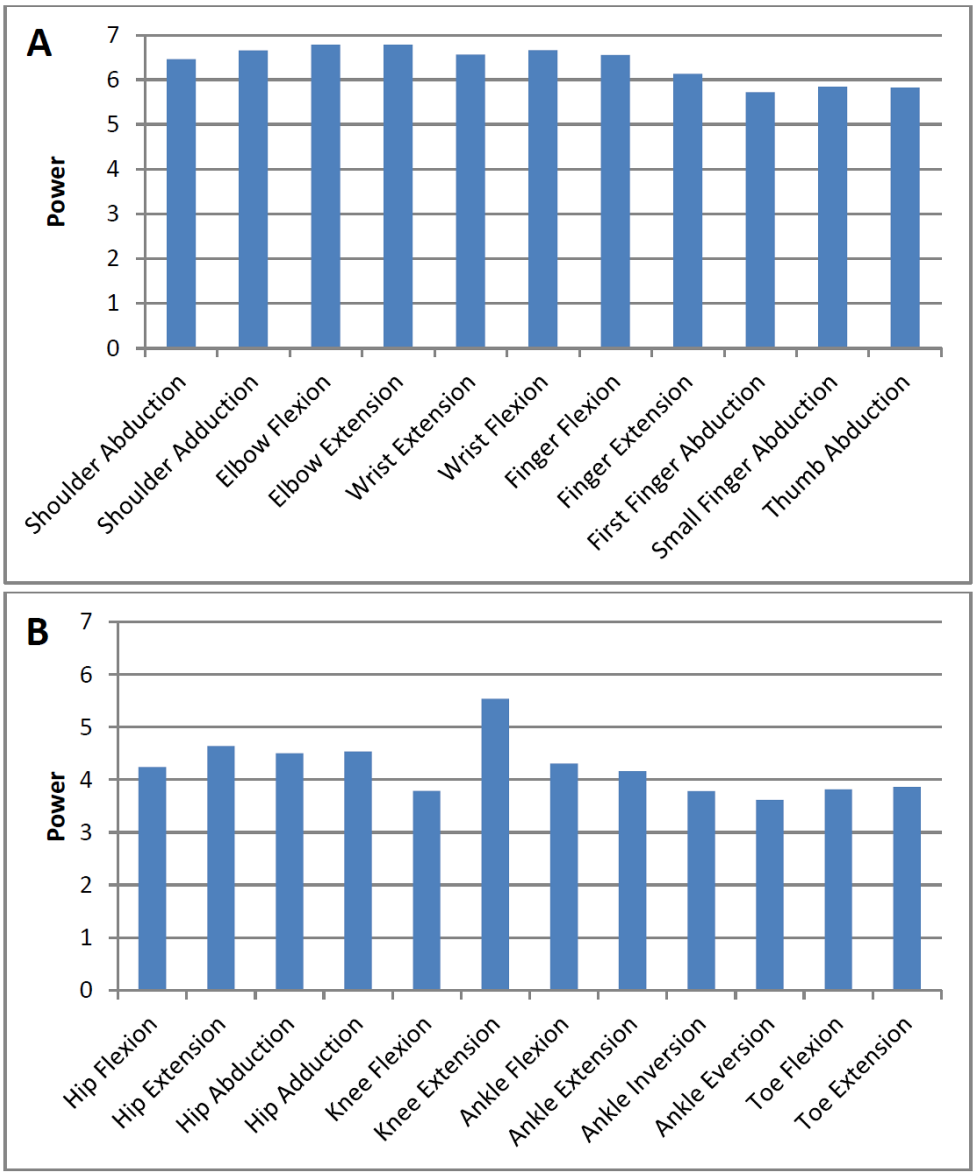


Figure 23: Mean muscle power values from SNE for (A) upper limb & (B) lower limb for EFACTS patients.

LL weakness is more severe than UL. For an explanation of the scale, see text.

Values for deep tendon reflexes are recorded in Table 17 representing bilateral measurements for up to 157 patients at baseline. The results are illustrated in Figure 24 showing percentages of the total number of patients examined.

Table 17: Deep tendon reflexes from the SNE for EFACTS patients at baseline

	Absent	Present only with reinforce-ment	Present but hyporeflexic	Normal	Hyper-reflexic	Hyper-reflexic with clonus
Biceps	282 (90.1)	2 (0.6)	11 (3.5)	12 (3.8)	6 (1.9)	0 (0)
Supinator	280 (89.5)	0 (0)	9 (2.9)	15 (4.8)	9 (2.9)	0 (0)
Triceps	140.5 (89.8)	2 (0.6)	12 (3.8)	12 (3.8)	6 (1.9)	0 (0)
Patellar	132 (85.7)	0 (0)	7 (2.3)	17 (5.5)	20 (6.5)	0 (0)
Ankle	139.5 (90.9)	2 (0.7)	6 (2.0)	13 (4.2)	7 (2.3)	0 (0)

	Absent	Present
Finger jerks	286 (92.3)	24 (7.7)
Hoffman reflex	297 (96.7)	10 (3.3)

	Mute	Flexor	Extensor	Withdrawal
Plantar	72 (23.8)	21 (7.0)	188 (62.3)	21 (7.0)

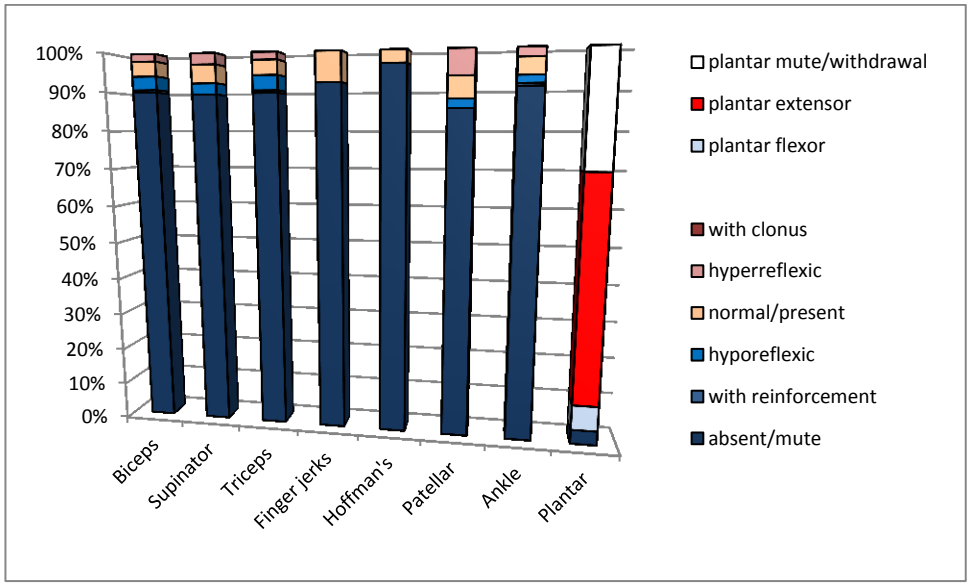


Figure 24: Distribution of deep tendon reflexes from SNE in EFACTS patients at baseline

The overwhelming majority of reflexes are absent in FRDA, whilst the plantar reflexes are usually extensor

Values for amyotrophy, muscle tone, sensory loss and skeletal foot abnormalities for the EFACTS patients as recorded in the SNE are shown in Table 18. The values

represent bilateral measurements for each patient using a series of semi-quantitative scales described in the Methods section at 2.2.2 above. The percentages represent the proportion of patients whose examination was recorded rather than of the whole cohort. As the SNE was time-consuming and not a core element of the EFACTS battery of assessments, this led to an incomplete data set. Sensation was assessed using a length-dependent scale described in the Methods section at 2.2.2 which is analyzed as a continuous variable with zero representing no sensory loss and each point representing sensory loss affecting a further joint. In the assessment of muscle tone, two patients were felt to have Gegenhalten rather than spasticity, defined as 'hypertonia characterized by involuntary variable resistance during passive movement…usually elicited by moving…rapidly from a contracted to a stretched position after instructing the patient not to resist' (Kreutzer *et al.* 2011). Figure 25 shows the proportion of patients with varying degrees of amyotrophy and spasticity. Figure 26 illustrates the extent of sensory loss covering the three modalities assessed. Figure 27 depicts the proportion of patients with various skeletal foot abnormalities seen in FRDA.

Table 18: Values for muscle atrophy, muscle tone, sensory loss and skeletal foot abnormalities from the SNE for EFACTS patients

		None	Mild	Moderate	Severe
Atrophy	**UL**	189 (66.3)	63 (22.1)	31 (10.9)	2 (0.7)
	LL	195 (73.6)	35 (13.2)	27 (10.2)	8 (3.0)

		Highly flaccid	Flaccid	Normal	Spastic	Highly spastic	Other
Tone	**UL**	2 (0.7)	4 (1.5)	228 (83.2)	34 (12.4)	6 (2.2)	0 (0)
	LL	6 (2.1)	3 (1.1)	206 (73.6)	45 (16.1)	18 (6.4)	2 (0.7)[a]

			Mean	SD	Mode	Range	*n*
Sensation	**UL**	**Pin prick**	1.13	1.8	0	0 – 7	284
		JPS	0.86	1.46	0	0 – 6	276
		Vibration	0.48	1.22	0	0 – 6	286
	LL	**Pin prick**	1.42	1.85	0	0 – 7	279
		JPS	1.69	1.47	2	0 – 5	253
		Vibration	2.33	1.94	0	0 – 6	288

cont…

	Absent	Mild	Moderate	Severe
Pes cavus	145 (49.3)	33 (11.2)	62 (21.0)	54 (18.4)
Talipes equinus	124 (44.3)	47 (16.8)	53 (18.9)	56 (20.0)
Talipes varus	145 (63.0)	28 (12.2)	26 (11.3)	31 (13.5)

UL=upper limb; LL=lower limb; JPS=joint position sense
[a]Gegenhalten

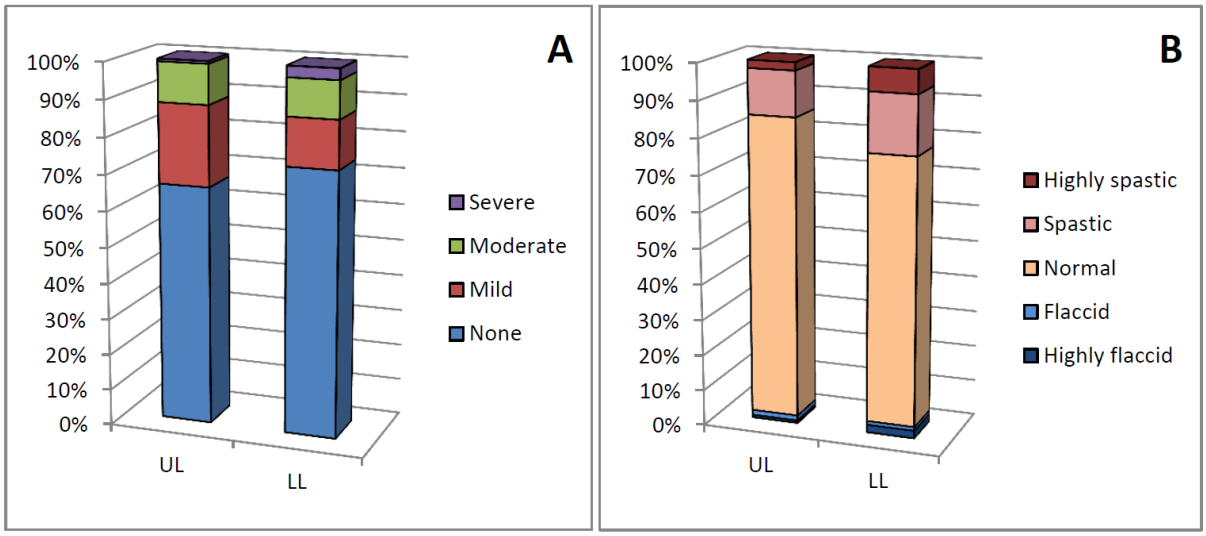


Figure 25: (A) Muscle atrophy and (B) spasticity as recorded in the SNE for EFACTS patients at baseline

Up to 40% of patients have some muscle atrophy, whilst up to 30% of patients have spasticity

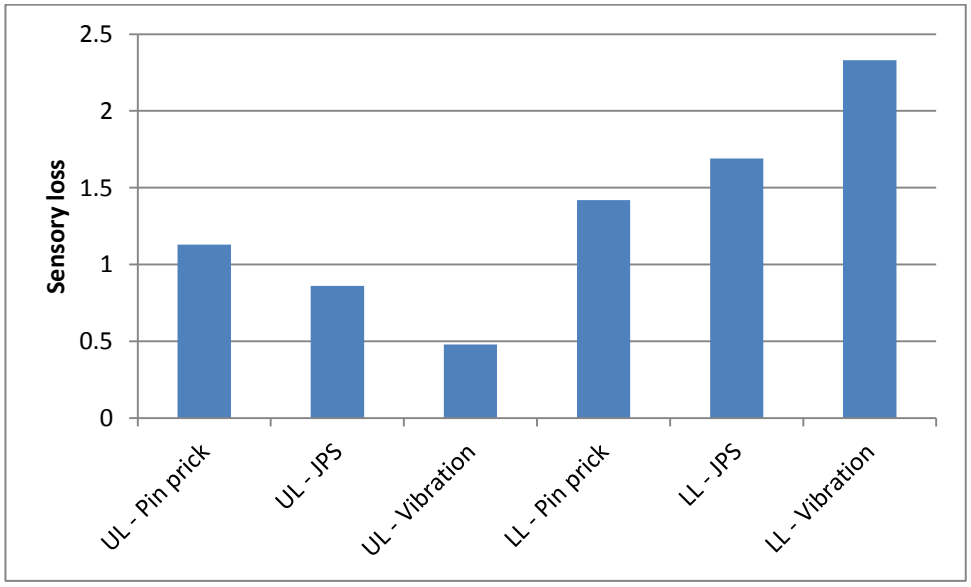


Figure 26: Extent of sensory loss as recorded in SNE for EFACTS patients at baseline.

The degree of sensory loss was defined on a length-dependent scale with higher numbers representing more proximal spread of the sensory loss. For a more detailed explanation of scale, see the Method at 2.2.2 above.

UL=upper limb; LL=lower limb; JPS=joint position sense

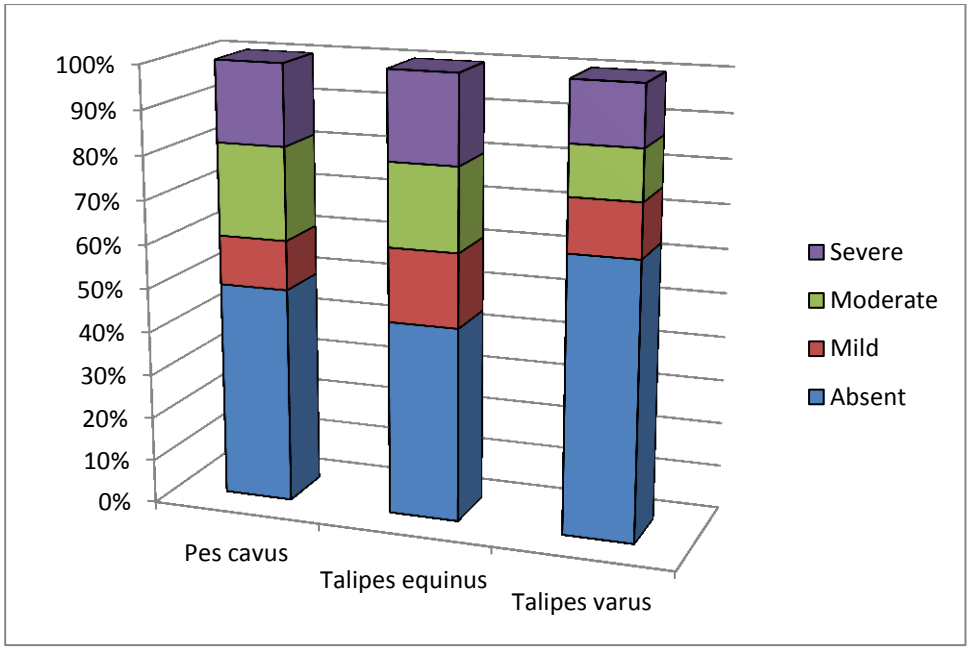


Figure 27: Skeletal foot abnormalities from SNE for EFACTS patients
Talipes equnius is the coomonest foot abnormality in FRDA, followed by pes cavus and then talipes varus

2.3.11 Disease Progression at Follow-Up Assessments

The aim of the clinical strand of the EFACTS project was to recruit patients with genetically confirmed FRDA, and then perform clinical assessments at baseline and on a yearly basis to provide detailed natural history data regarding progression of the condition which would inform the planning of clinical trials and provide prognostic information for patients and clinicians. Figure 28 shows the passage of patients through the UK portion of the study. 167 genetically proven FRDA patients were recruited between 1 August 2011 and 1 August 2014 and underwent baseline (BL) assessment as described above. Of these, 125 were seen for follow-up assessment at year 1 ±3 months (FU1). Six patients were lost to follow-up : one died; two were known to have moved abroad; contact was lost with a further three despite repeated attempts to communicate with them. Seventeen were still awaiting assessment at the time of data collection for this thesis (all from phase 2, those patients recruited later in the study after the recruitment of the initial core phase 1 sample). Nineteen patients therefore missed the year 1 assessment, of which 16 were subsequently reassessed at year 2 and hence not lost to the study. In total 116 patients were seen at the year 2 follow-up assessment (FU2). Twenty-seven were therefore still awaiting assessment at

the time of data collection for this thesis, all patients who had entered toward the end of phase 1 recruitment.

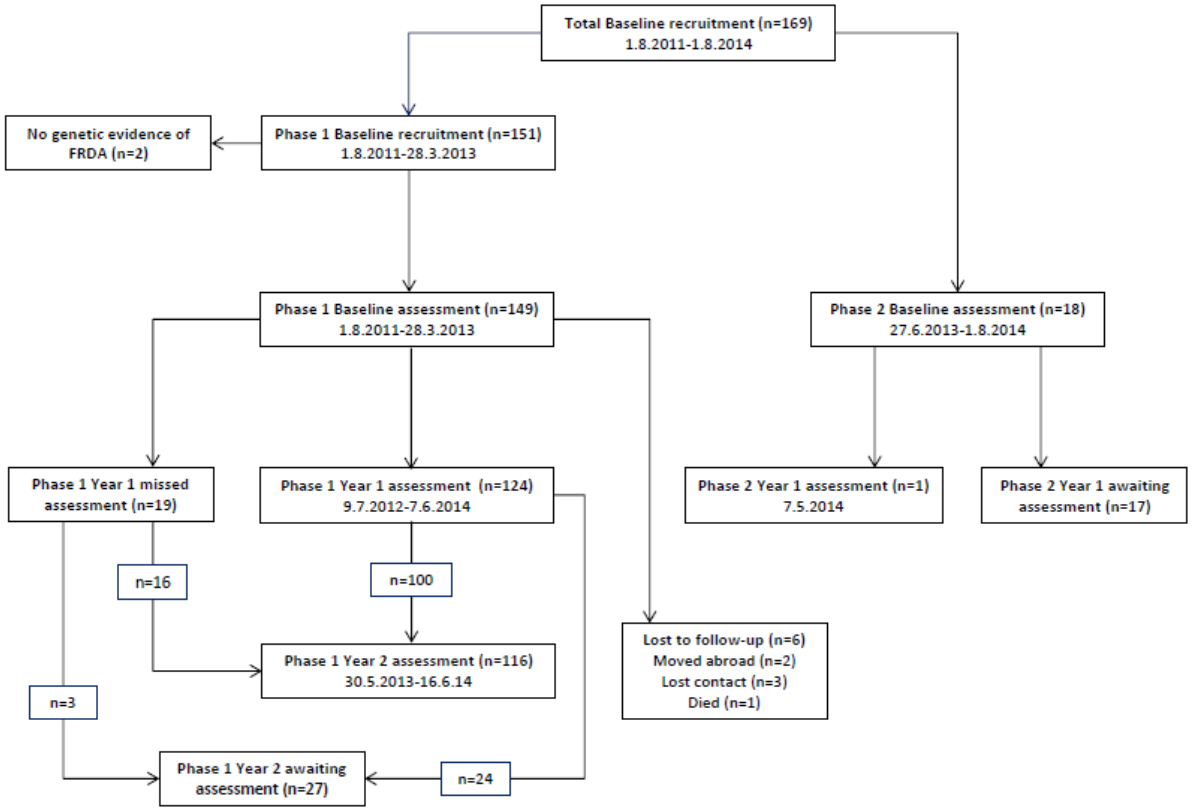


Figure 28: Study profile of patients in UK contribution to EFACTS

2.3.11.1 SARA

Results are recorded for the SARA for 166 patients at BL, 122 at FU1 and 116 at FU2. Figure 29 shows the total SARA data for all patients passing through the study. Although this clearly shows that patients do not follow a simple pattern of uniformly deteriorating year after year, because of the large number of patients in the study and their variable outcome, this form of representation is not easy to interpret and so is not repeated for subsequent measures.

The mean, SD and range of total SARA data are recorded in Table 19. Of note, 27 patients were still awaiting FU2 assessment at the time of data collection for the thesis. In addition, the results of those patients seen at BL but not FU1 are recorded separately ('BL patients without FU1 assessment'), and also those patients seen at BL who were subsequently seen at FU1 ('BL patients with FU1 assessment'). The former group provide information about whether those individuals who were not seen at FU1

differed from the group who were followed-up. The latter group provide a directly comparable group of patients seen both at BL and FU1 which can be analyzed using a paired t-test. Analogous BL data are presented with respect to FU2, the maximal period of follow-up in this study.

Of the 116 patients for whom both BL and FU2 data were available, 31 (26.7%) improved, 14 (12.1%) showed no change in total SARA and 71 (61.2%) deteriorated. Explanations of why patients should seem to improve in a progressive degenerative condition are explored extensively in subsequent pages and discussed further at the end of the Discussion of this Chapter at 2.4.6 below. Figure 31(A) shows the changes in the individual total SARA values for each patient ranked in order of change with positive values indicating deterioration. The BL total SARA for those individuals who improved was 27.0±8.0 (4-34), for those without change in total SARA 32.9±7.7 (12-40), and those who deteriorated 19.7±9.8. These differences in BL total SARA are significant (1-way ANOVA, $F=16.0$, $p<0.0005$). When each group is compared separately, the differences are also significant (improved vs. no change, independent samples t-test, mean difference 5.9±2.6, 95% CI 0.7-11.0, $t=2.3$, $p=0.027$; no change vs. deteriorated, mean difference 13.2±2.4, 95% CI 8.3-18.1, $t=5.6$, $p<0.0005$; improved vs. deteriorated, mean difference 7.4±1.9, 95% CI 3.7-11.1, $t=4.0$, $p<0.0005$). These data suggest that individuals with milder disease tend to progress more than do those with more severe disease, as measured by the total SARA. It is possible, however, that this represents a 'ceiling effect' in the SARA, as those individuals with high BL total SARA scores have less scope within the scale to deteriorate as there is a maximal score. Indeed, it has already been observed on the analysis of the BL data in Section 2.3.7 above, that many FRDA patients occupy the highest scores of many of the SARA subscores, particularly those reflecting LL function.

Table 19: Total SARA for EFACTS patients at BL, FU1 and FU2

	Mean	SD	Range	*n*
BL (all data)	22.5	10.0	1.5 – 40	166
FU1 (all data)	24.4	9.7	2.5 – 40	122
FU2 (all data)	24.6	9.5	2.5 – 40	116
BL (patients with FU1 assessment)	23.5	10.0	1.5 - 40	122

BL (patients without FU1 assessment)	19.7	10.2	2.5 - 34	44
BL (patients with FU2 assessment)	23.2	10.2	1.5 – 40	116
BL (patients without FU2 assessment)	20.7	9.4	2.5 – 34	50

Figure 30(A) shows the change in total SARA from BL to FU1 and FU2. However, as shown above, there are large numbers of missing data at the FU1 and FU2 visits. It is statistically problematical comparing data containing mixed paired and unpaired values, that is to say, the complete data sets including missing data. For those individuals for whom paired total SARA data were available, there were significant differences between BL and FU1 (paired t-test, mean difference 0.91±2.69, 95% CI 0.43-1.40, t=3.76, p<0.0005) and between BL and FU2 (paired t-test, mean difference 1.33±3.14, 95% CI 0.75-1.91, t=4.57, p<0.0005), with both deteriorating.

There was a significant difference between the total SARA at BL for individuals who were subsequently seen at FU1 compared to those that were not (independent samples t-test, mean difference 3.7±1.7, 95% CI 0.3-7.2, t=2.1, p=0.033). Perhaps surprisingly, those who did not return for FU1 assessment had significantly lower BL total SARA, suggesting that the explanation of their failure to return for follow-up assessment was not because of increased disability. Indeed, it may be that these individuals who were less severely affected found it more difficult to return because they were more likely to be employed or lived further away. However, there was no significant difference in BL total SARA between those individuals seen at FU2 and those that were not (mean difference 2.52±1.69, 95% CI -0.82-5.86, t=1.48, p=0.139) suggesting that those patients who were not assessed at FU2 were not atypical of the group. This may be because at the point of data collection for the thesis, only six patients were truly lost to the project and the remaining 50 missing patients were still awaiting FU2 assessment rather than having formally missed their assessment in the context of the study. This is more likely to be a stochastic rather than systematically biased effect. For this reason, subsequent analysis will concentrate on the change from BL to FU2. This is obviously also the maximal period of follow-up in this study which

would therefore be expected to be associated with maximal change, as is seen for the total SARA.

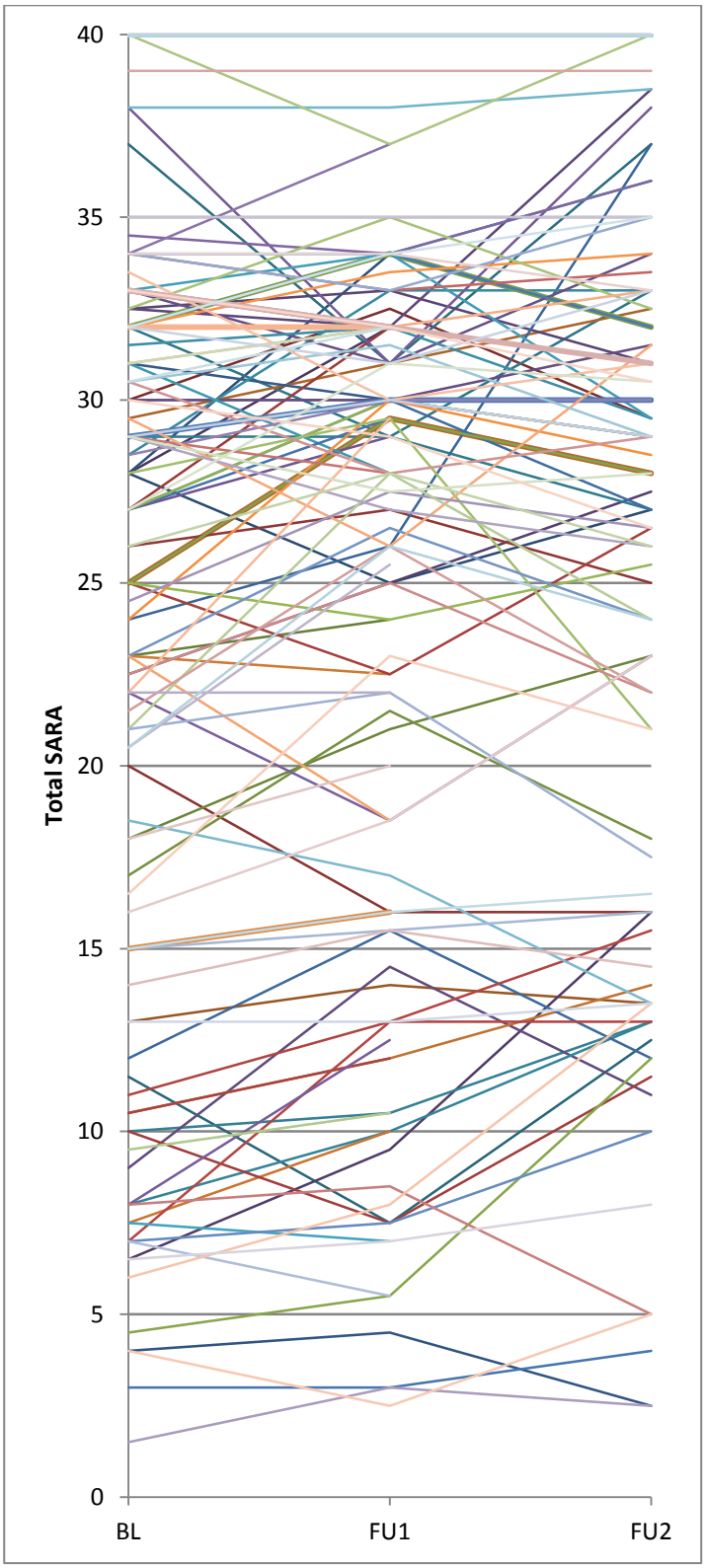


Figure 29: Total SARA values for individual EFACTS patients for BL, FU1 & FU2 (all data)

In order to try and minimize the ceiling effect of the SARA caused by the higher scores for the LL measures, the data were subdivided into three subcategories : upper limb (UL), lower limb (LL) and 'other'. The UL SARA included the subscores for (i) finger chase test (ii) nose-finger test and (iii) fast alternating hand movements (maximum core 12). The LL SARA included the subscores for (i) gait (ii) stance and (iii) heel-shin test (maximum score 18). The 'other' SARA included the two remaining subscores for (i) sitting and (ii) speech (maximum score 10). Table 20 shows the results for these three subcategories at BL, FU1 and FU2. These are displayed graphically in Figure 30(B) & (D). Table 20 also shows the results of the paired samples t-test comparing UL, LL and 'other' SARA results between BL, FU1 and FU2 for those participants for whom paired data were available. This shows that the only significant differences were in fact for the LL and 'other' subcategories, all of which survived Bonferroni correction for multiple testing. These results suggest that although there is high occupancy of the higher values for the constituent LL subscores of the SARA (see Figure 16), progression as measured by the SARA still seems to be driven by an increase in the LL subscores (as well as speech and sitting). Figure 31(B),(C) & (D), which show the changes in SARA subcategory score for each individual patient, illustrate this point more clearly. For the UL subcategory, only 39 patients (33.6%) deteriorated. 24 (20.7%) remained unchanged and 53 (45.7%) actually improved. By contrast, for the LL subcategory, 48 patients (41.4%) deteriorated, 56 (48.3%) remained unchanged and only 12 (10.3%) improved. For the 'other' subcategory, 42 (36.2%) deteriorated, 57 (49.1%) remained unchanged and 17 (14.7%) improved. Although numerically more patients improved than deteriorated on the UL subcategory, as Table 20 and Figure 30 show, the mean UL SARA increased (*ie* deteriorated) between BL and both FU1 and FU2. These differences were not, however, statistically different. As Figure 31(B) shows, the maximum improvement in the UL SARA was 4 points, whereas the maximum deterioration was 10 points, and so although a smaller number deteriorated than improved, they deteriorated to a greater extent, making the mean change tend toward deterioration.

Table 20: SARA scores for BL, FU1 & FU2 visits divided into UL, LL & 'other' subcategories

Subcategory	Visit	Mean	SD	n
UL SARA	BL (all data)	4.9	2.9	166
	FU1 (all data)	5.3	2.9	122
	FU2 (all data)	5.2	3.1	116
	BL (patients with FU1 assessment)	5.1	3.0	122
	BL (patients with FU2 assessment)	5.2	3.0	116
LL SARA	BL (all data)	13.5	5.5	166
	FU1 (all data)	14.3	5.1	122
	FU2 (all data)	14.7	4.8	116
	BL (patients with FU1 assessment)	13.8	5.5	122
	BL (patients with FU2 assessment)	13.6	5.5	116
'Other' SARA	BL (all data)	4.1	2.7	166
	FU1 (all data)	4.8	2.5	122
	FU2 (all data)	4.7	2.6	116
	BL (patients with FU1 assessment)	4.2	2.7	122
	BL (patients with FU2 assessment)	4.3	2.8	116

Subcategory	Visits	Mean difference	95% CI	t	*p*†
UL SARA	**BL-FU1**	0.28±1.79	-0.04-0.40	1.71	0.088
	BL-FU2	0.04±1.95	-0.32-0.40	0.24	0.813
LL SARA	**BL-FU1**	0.52±1.86	0.19-0.85	3.09	0.002
	BL-FU2	1.06±2.27	0.64-1.48	5.04	<0.0005
'Other' SARA	**BL-FU1**	0.52±1.31	0.28-0.75	4.33	<0.0005
	BL-FU2	0.43±1.22	0.21-0.65	3.81	<0.0005

†Bonferroni correction reduces significant p value to 0.05/6=0.0083

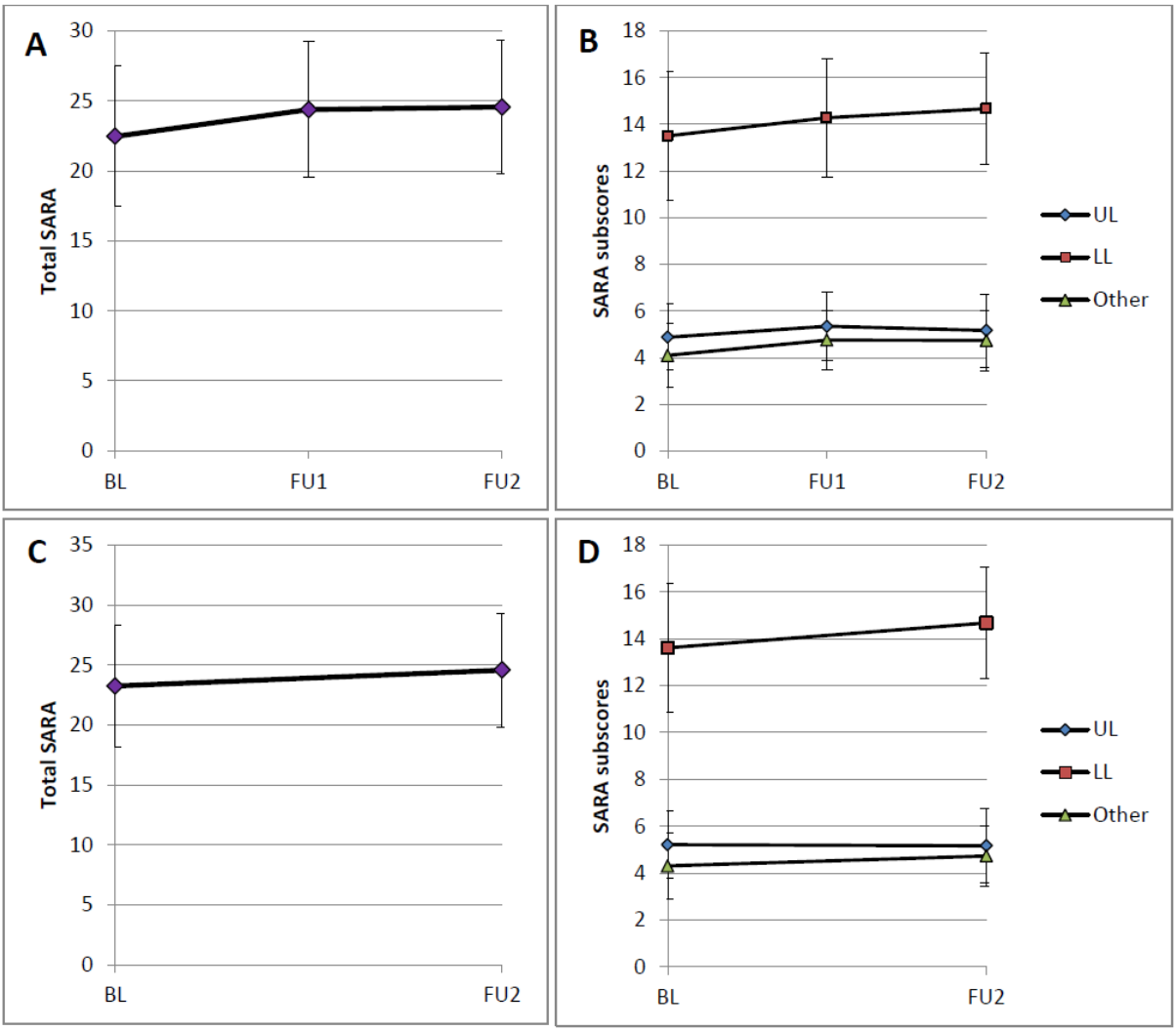


Figure 30: Mean SARA values between BL, FU1 & FU2

(A) Total SARA & (B) SARA subcategories for all available data (BL *n*=166, FU1 *n*=122, FU2 *n*=116). (C) Total SARA & (D) SARA subcategories for complete datasets for BL & FU2 only (*n*=116). Error bars=SD.

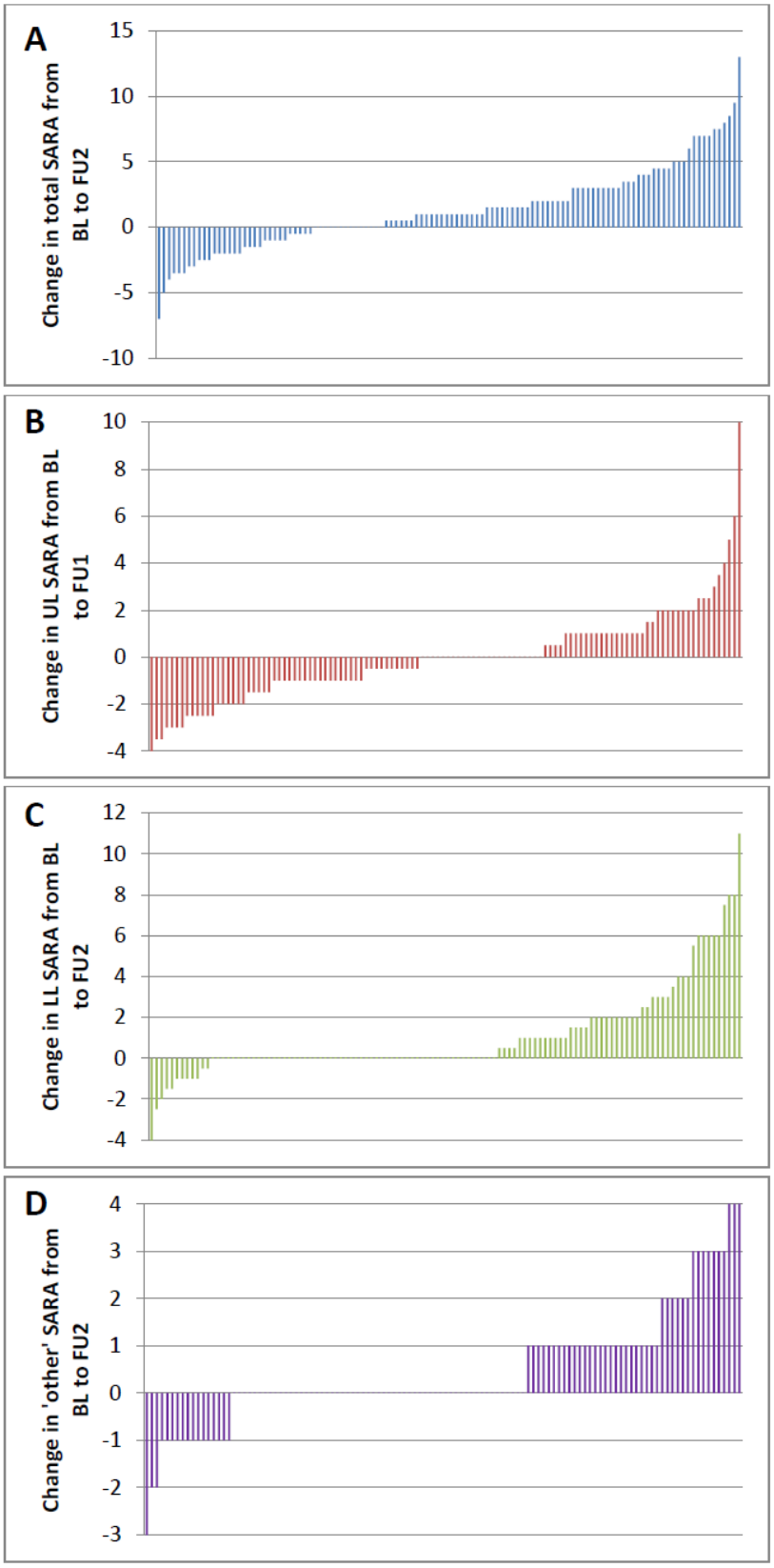


Figure 31: Change in (A) total SARA (B) UL SARA (C) LL SARA and (D) 'other' SARA. Each bar represents change in SARA from BL to FU2 for an individual patient (*n*=116), ranked by change from negative (on left) to positive (on right). Negative values represent improvement, positive values deterioration and zero, no change.

Figure 32 attempts to investigate whether there are any underlying factors which predict which patients will progress more rapidly. As can be seen, there was no correlation between rate of progression and GAA1 size, age at onset, age at examination, disease duration and BL total SARA. This observation has been made previously in a smaller sample (Marelli *et al.* 2011). In addition, no significant difference was seen in SARA disease progression between males (*n*=47) and females (*n*=69) (Mann-Whitney U-test, U=1509.5, p=0.528).

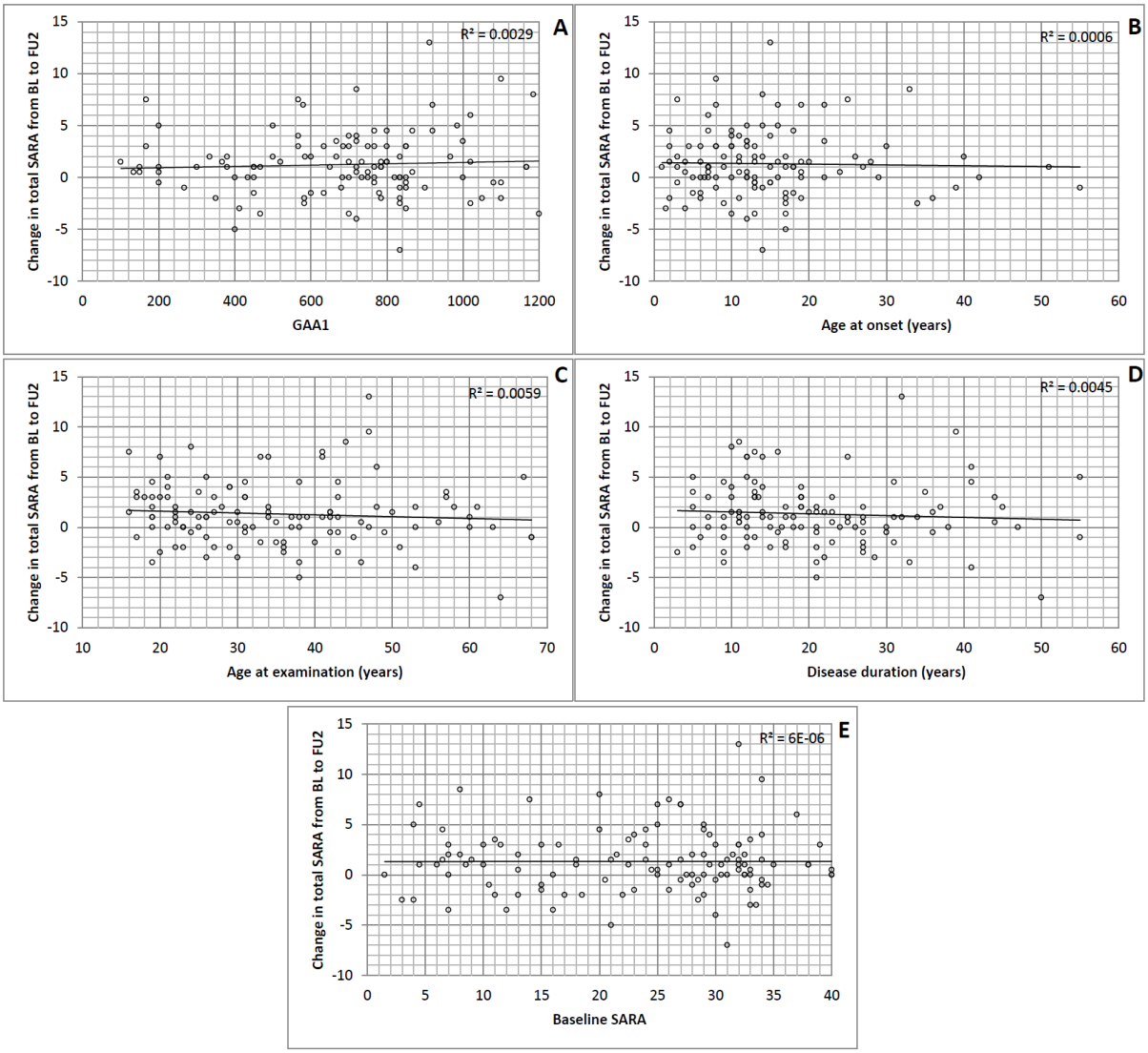


Figure 32: Relationship between change in total SARA from BL to FU2 and (A) GAA size, (B) age at onset, (C) age at examination, (D) disease duration, and (E) BL total SARA

The change in SARA is independent of these 5 measures

2.3.11.2 INAS

INAS count data were available for 166 patients at BL, 124 at FU1 and 116 at FU2. These are shown in Table 21 and illustrated graphically in Figure 33. There were no significant differences between BL and FU1 (paired t-test, mean difference 0.17±1.44, 95% CI -0.09-0.42, t=1.314, p=0.191) or between BL and FU2 (mean difference 0.09±1.43, 95% CI -0.18-0.35, t=0.647, p=0.519). Between BL and FU2, 38 patients (32.8%) improved with regard to INAS count, 25 (30.2%) remained unchanged (30.2%) and 43 (37.0%) deteriorated. Thus, the INAS count failed to capture change in non-ataxic signs and symptoms in the study population over a 2 year period.

Table 21: INAS count values for EFACTS patients at BL, FU1 & FU2

	Mean	SD	Range	*n*
BL (all data)	5.0	1.6	2 - 9	166
FU1 (all data)	4.9	1.6	1 - 8	124
FU2 (all data)	5.0	1.4	2 - 8	116
BL (patients with FU1 assessment)	5.0	1.6	2 - 9	124
BL (patients with FU2 assessment)	5.0	1.6	2 - 9	116

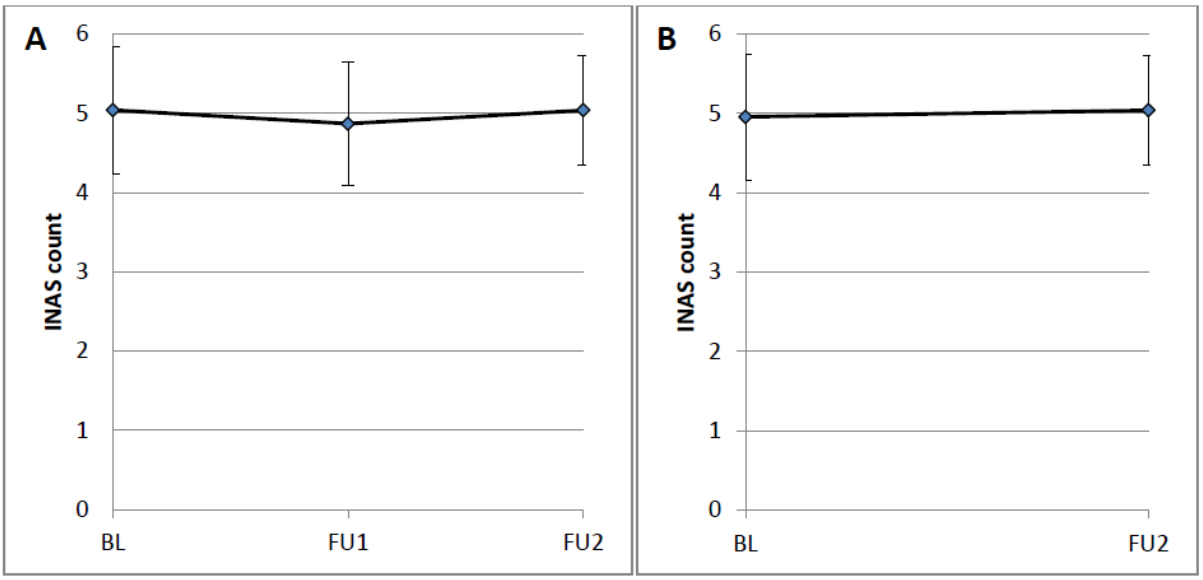


Figure 33: Mean INAS count between BL, FU1 and FU2
(A) for all available data (BL *n*=166, FU1 *n*=124, FU2 *n*=116) (B) for complete datasets for BL to FU2 only (*n*=116). Error bars=SD.

2.3.11.3 ADL

Total ADL data were available for 162 patients at BL, 122 at FU1 and 113 at FU2. The data are shown in Table 22 and graphically in Figure 34. The total ADL detected change at both FU1 (paired t-test, mean difference 1.07±3.67, 95% CI 0.42-1.73, t=3.233, p=0.002) and FU2 (mean difference 2.04±3.22, 95% CI 1.45-2.64, t=6.756, p<0.0005). Between BL and FU2, 24 patients (21.2%) improved as measured by the total ADL, no change was recorded in 14 (12.4%) and 75 (66.4%) deteriorated.

Table 22: Total ADL for EFACTS patients at BL, FU1 & FU2

	Mean	SD	Range	*n*
BL (all data)	15.4	8.3	1 - 35	162
FU1 (all data)	17.1	8.6	1 - 36	122
FU2 (all data)	17.9	8.6	1 – 36	113
BL (patients with FU1 assessment)	16.1	8.1	1 - 35	122
BL (patients with FU2 assessment)	15.9	8.6	1-35	113

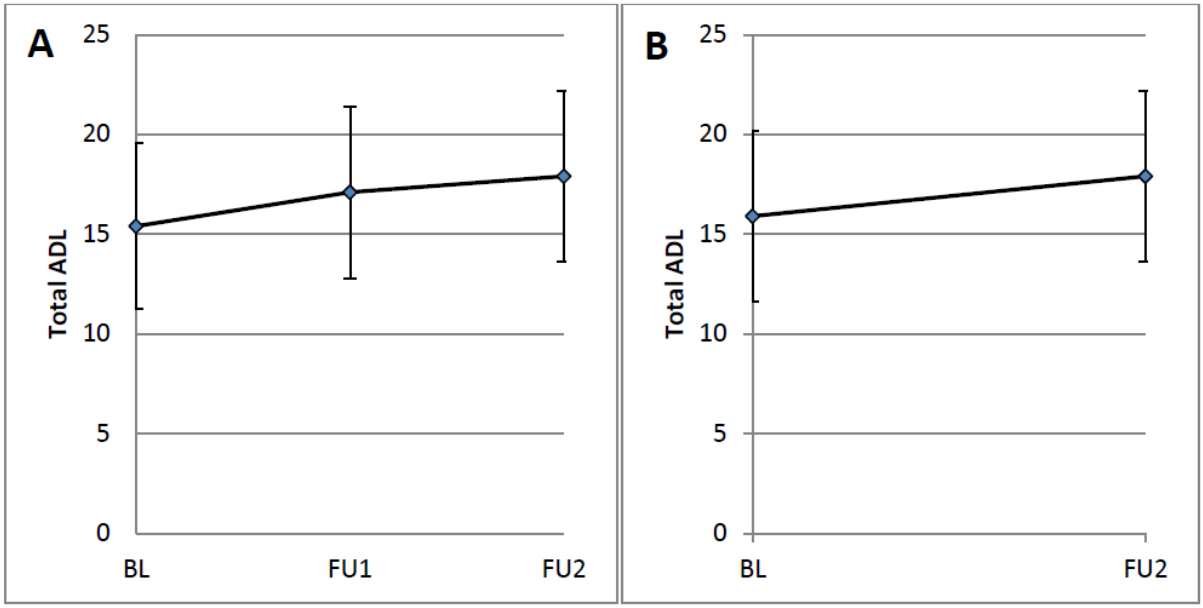


Figure 34: Mean total ADL between BL, FU1 and FU2
(A) for all available data (BL *n*=162, FU1 *n*=122, FU2 *n*=113) (B) for complete datasets for BL to FU2 only (*n*=113). Error bars=SD.

2.3.11.4 SDFS

SDFS data were available for 167 individuals at BL, 138 at FU1 and 129 at FU2. Table 23 shows the mean, SD and ranges of these data. However, they are ordinal, heavily

skewed to the highest value and there are only seven possible responses (the seven disability stages of the SDFS). Table 23 therefore also shows the frequency and proportion of patients by SDFS stage. Figure 35 shows that the mean SDFS increases from BL to FU1 and FU2, and a greater proportion of patients pass into the higher disability stages. For the individuals for whom matched data were available, there was a significant increase in SDFS stage between BL and FU1 (Wilcoxon signed ranks test, Z=-2.874, p=0.004) and between BL and FU2 (Z=-4.673, p<0.0005).

Table 23: SDFS for EFACTS patients for BL, FU1 & FU2

	Mean	SD	Range	*n*
BL (all data)	5.0	1.3	1 - 7	167
FU1 (all data)	5.2	1.2	1 - 7	138
FU2 (all data)	5.3	1.0	2 - 7	129
BL (patients with FU1 assessment)*	5.1	1.3	1 - 7	138
BL (patients with FU2 assessment)**	5.0	1.4	1 - 7	129

SDFS	BL	FU1	FU2	BL with FU1*	BL with FU2**
Stage 1	1 (0.6)	1 (0.7)	0 (0)	1 (0.7)	1 (0.8)
Stage 2	8 (4.8)	3 (2.2)	2 (1.6)	4 (2.9)	6 (4.7)
Stage 3	25 (15.0)	15 (10.9)	11 (8.5)	20 (14.5)	19 (14.7)
Stage 4	15 (9.0)	11 (8.0)	11 (8.5)	12 (8.7)	10 (7.8)
Stage 5	23 (13.8)	24 (17.4)	24 (18.6)	20 (14.5)	17 (13.2)
Stage 6	94 (56.3)	83 (60.1)	79 (61.2)	80 (58.0)	75 (58.1)
Stage 7	1 (0.6)	1 (0.7)	2 (1.6)	1 (0.7)	1 (0.8)
Total	167	138	129	138	129

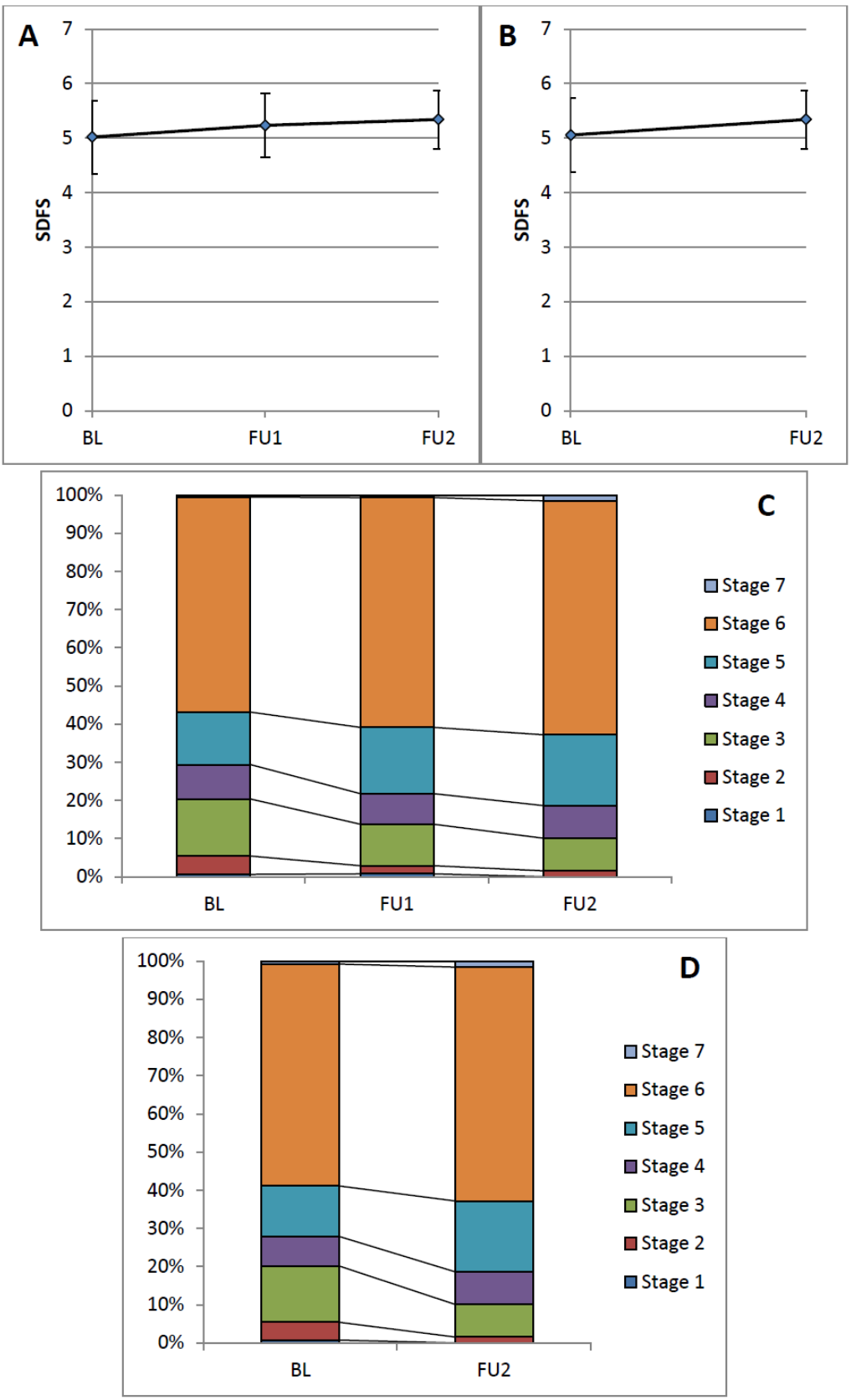


Figure 35: SDFS for BL, FU1 & FU2 (A) & (C) for all available data (BL *n*=167, FU1 *n*=138, FU2 *n*=129), (B) & (D) for complete datasets (*n*=129), (A) & (B) for mean values (error bars=SD), (C) & (D) proportion of patients with different SDFS stage

2.4 Discussion

The data presented above represent the largest natural history study ever undertaken of Friedreich's ataxia in the UK, and the cohort described forms the largest contributing centre to the largest natural history study ever published of FRDA (Reetz *et al.* 2015). Since the discovery of the *FXN* gene in 1996, there has been a paucity of large natural history studies in which the patients have a genetic as well as clinical diagnosis, with a single early study of 140 patients (Dürr *et al.* 1996) and most subsequent studies involving fewer than 60 patients (see Chapter 1). No study has assessed the clinical features of FRDA in such detail including a range of formal clinical assessment tools. Whilst there was an inevitable predilection of patients from the South-east of England, great effort was made to include patients from all over the British Isles including those from the Republic of Ireland. This therefore represents the largest and most detailed study of FRDA in the British Isles which will provide invaluable natural history data to be used in the planning and execution of interventional trials in FRDA, and essential information to be used in guiding the diagnostic process and counselling patients regarding prognosis. It has also permitted the compilation of a database of patients who are willing to participate in clinical trials which has already contributed to one of the first studies of an epigenetic therapeutic agent in FRDA. Significantly, preliminary evidence has shown increased levels of frataxin expression sufficient to warrant further study (Libri *et al.* 2014). More widely, the central clinical registry of EFACTS data allied to a biological sample repository will allow genetic, biomarker and other basic science projects to investigate underlying pathological processes.

The very large size of the study has allowed it to encompass patients with a wide range of presentations from full mobility and minimal disability to complete dependence and confinement to bed. The latter necessitated a number of home visits to ensure these patients were included. Patients were seen with age at onset from 1 to 55, embracing the early, classical, late and very late onset phenotypes. Patients were also seen throughout the course of their disease from age 16 to 68 with disease duration of between 3 and 55 years. Patients with GAA expansions of between 100 and 1500

repeats were enrolled in the study providing a wide range of disease severities. Most patients recruited to the study already had a genetic diagnosis but two had to be excluded after inclusion as the genetic test failed to confirm the diagnosis: no alternative genetic diagnosis has since been found in either case.

The systematic prospective nature of the clinical assessment is a major advantage of this study ensuring comparability of the results from patient to patient. Furthermore, only two clinicians saw patients within this study and the vast majority of the assessments were undertaken by the author. Thus, there was minimal inter-rater variability between patients, and between baseline and subsequent assessments. The systematic central storage of data allowed very rigorous and independent management of data quality, and permits 'data mining' and downloading of easily statistically analyzable data. Whilst the centrally prescribed nature of the battery of assessments undertaken throughout all participating groups is a major advantage of the project, certain features were not captured well. Examples of visual and hearing impairment have already been discussed in the text. Certain other features were not assessed, such as imaging and cognition. The latter has been addressed as the patients currently undergo the Montreal Cognitive Assessment (MoCA). Furthermore, GAA size data (provided by the Université Libre de Bruxelles) were only available for 147 of the participants at the time of thesis production

Whilst this thesis concentrated on analysis of the baseline data, progression data were available for more than 100 patients over a 2 year period. These data are vital in planning interventional trials and can only be obtained from large, well-funded, well-structured studies. Retention rates were generally good and the patients have continued to attend after the point of data collection for this thesis, allowing the possibility of long-term progression data to be collected. The necessity to acquire the data for thesis production during an ongoing project meant that an arbitrary cut-off of 1 August 2014 had to be made and the follow-up data are consequently incomplete: seventeen patients were awaiting their first follow-up assessment and twenty-seven their second when the data were downloaded for analysis. More seriously, nineteen patients missed their first follow-up assessment and so will skip directly to the second follow-up assessment.

Whilst on the one hand an advantage, the sheer size of the study and the profusion of data collected pose problems of which data to analyze and how to present them concisely and in a readily comprehensible fashion. Only the reader can decide if this endeavour has been successful.

2.4.1 Clinical Features

There has been no consistent gender predominance found previously, and the ratio of 1:1.3 of males to females in the present study is broadly in keeping with previous studies. Although the UK is a multi-ethnic nation and patients were recruited who were born in 15 different countries across all six continents, it is significant that no patients of Afro-Caribbean origin were found. This is in keeping with previous studies which have found FRDA patients of European, North African, Middle Eastern and Indian origin but not from sub-Saharan Africa, Native America, China, Japan or South-East Asia (Labuda *et al.* 2000). It is likely that whilst the origin of the GAA repeat sequence was somewhere between Old and New World Monkeys (González-Cabo *et al.* 1999), its pathological expansion did not take place until after the African diaspora.

The demographics details reveal some of the social consequences of FRDA. Patients with FRDA typically manage high levels of education with more than 30% having a university degree and more than 50% having a post-school qualification. Approximately one third of the cohort are in employment. Just over 30% are in a relationship and just under 30% have children (see Table 3).

The cohort contained 5% of compound heterozygotes which is comparable to previous studies. The commonest point mutation in this cohort is the p.Gly130Val missense mutation which is known to be common in Caucasian populations (Bidichandani *et al.* 1997). The cohort also includes a patient bearing a macrodeletion which are extremely rare and have only recently been described (Zühlke *et al.* 2004). These cases will be described in greater detail in Chapter 3. For those individuals bearing two GAA expansions, the shorter of the two are distributed normally around a modal value of 700 repeats and extending up to a maximum value of 1200. The longer of the two are distributed slightly more unevenly around a median value of 967 repeats and

extending up to 1500 repeats. These values are comparable with previous reports (Dürr *et al*. 1996).

The study recruited patients with a wide variety of ages, disease durations and ages at onset, including patients with very late onset and long duration (see Figure 8 and Figure 9). A criticism of this cohort is that no children were included, an issue which is being addressed in the wider EFACTS cohort. The distribution of ages at onset is heavily skewed toward early onset. 148 of the patients (88.6%) had onset below the age of 25, in keeping with the Harding diagnostic criteria (Harding 1981). Only five patients (3.0%) had onset at or after age 40 putting them in the category of very late onset FRDA (see Figure 11). The first symptoms are almost always gait instability, falls, clumsiness or complications of poor manual dexterity, with gait instability forming by far the largest proportion. Scoliosis is the only non-neurological symptom which is found at presentation in a significant proportion of patients (9.6%). Presentation with diabetes or cardiomyopathy is very rare (see Figure 13).

Progression in FRDA is slow but steady, predominantly affecting gait and lower limb function. Well over half of the cohort were wheelchair-bound by the time of assessment and less than 1% reported no effect on mobility (see Table 7). Nearly 80% used some aid to walking. Of those who are confined to a wheelchair, the median age was 19, with one patient requiring a wheelchair from the age of five. 26.4% of the cohort required a wheelchair before the age of 20, and 43.7% before the age of 30.

The SNE reveals in far greater detail the pattern of impairment seen in FRDA. Upper limb power remains very well preserved, particularly proximally, with a large proportion of patients having full power. Significant lower limb weakness is much more common, both proximally and distally (see Figure 23). It has previously been noted that there is slowly progressive and symmetrical weakness mainly affecting the lower limbs, especially the muscles of the pelvic girdle. In a longitudinal study over 13 years of 33 patients with FRDA, weakness of hip extension was by far the commonest first location of weakness, with pelvic girdle muscle power declining by 2.1% per year. Average lower limb power was approximately 70% of normal by the time a wheelchair was first used, and 56% when the patients were completely unable to walk. By

contrast, upper limb and truncal power were well preserved until late in the disease process with an overall strength of approximately 80% of normal (Beauchamp *et al.* 1995). Similarly, in a cross-sectional study of 12 children with FRDA, lower limb weakness was significantly greater than upper limb weakness, and in the legs, proximal weakness was significantly greater than distal (Sival *et al.* 2011). Concordant with these clinical observations, neuropathological examination of the spinal cord shows that the dorsal root ganglia and dorsal columns are particularly affected. There is reduced calibre of the spinal cord at all levels but more pronounced in the thoracic region. There is loss of myelin and axons in the corticospinal tracts, dorsal columns and spinocerebellar tracts (Koeppen 2011). The pathology in the sensory pathways is dominated by dorsal root ganglial and spinal degeneration. Far less is known about the pathology of the motor pathways, but it seems likely that the greatest involvement is in the lower thoracic and lumbar spine giving rise to predominantly proximal as well as distal weakness in the lower limbs.

The deep tendon reflexes are characteristically absent, with more than 85% of all reflexes studied, absent. Brisk reflexes are extremely rare. Extensor plantar responses are seen in over 60% of cases and flexor responses in less than 10% (see Table 17).

Muscle atrophy is seen in around 30% of patients, particularly in the hands and feet, although this is sometimes difficult to discern because of poor growth in young-onset patients. This topic is explored in Section 2.4.3 below. Muscle tone is predominantly normal. Around 15-20% of patients show significant spasticity, more common in the lower limbs than the upper. In keeping with the frequent areflexia, sensory loss is common, extensive and debilitating. Posterior column modalities predominate, but pin prick sensation is also significantly affected, particularly in the ULs (see Table 18).

Although 43% of the patients in the series have some degree of cardiomyopathy, patients with FRDA rarely have fulminant cardiac failure. Indeed, based on the reference values given in Table 10, 78.0% of patients had normal IVSd; only 2.2% had moderate or severe IVSd thickening. 79.1% of patients had normal LVPWd and only 4.7% moderate or severe thickening. 86.2% had normal LVEF with only 5.7% having moderate or severe LV impairment. Repolarization changes (69.4%) and to a lesser

extent voltage criteria for LVH (40.0%) on ECG are, however, much more frequently observed. This is significant as these changes are evident from early in the disease process and are not typically seen in other early-onset ataxic disorders (in which cardiomyopathy is not a feature). It therefore acts as a diagnostic clue. Although only 3.4% of the ECGs seen as part of the study showed any arrhythmia, 15.6% of the patients had a history of cardiac arrhythmia including both atrial fibrillation and supraventricular tachycardia. Ischaemic heart disease is very rare in FRDA (1.2%). However, to the uninitiated eye, the repolarization changes seen in FRDA, such as inferolateral T wave inversion, may resemble ischaemic changes. Compounding this, it has recently been reported that 46.9% of FRDA patients who were asymptomatic from a cardiac point of view, have cardiac troponin I levels above the 99th percentile of normal, and 16.3% have values typically seen during acute myocardial infarction (Friedman *et al.* 2013). Thus, in assessing a patient with FRDA, it is essential to understand the normal appearance of the ECG.

In keeping with previous studies, the prevalence of diabetes mellitus in the cohort was low (8.9%). Previously reported rates of scoliosis have varied greatly, presumably depending on the degree of deformity counted as constituting significant scoliosis. Although 69.5% of the patients in the study showed some evidence of scoliosis, only 30% of these had moderate to severe scoliosis.

The study highlights certain features which have not previously attracted much attention. Ptosis was seen in 16.6% of the patients. Talipes equinus and varus abnormalities are as common as pes cavus and occur in around 50% of patients. Visual and hearing impairment are common. Probably a third of patients have hearing loss attributable to FRDA. These often coexist. Of the 24 patients thought to have visual loss attributable to FRDA, 14 also had hearing loss (58%). Unfortunately, these features were not well-captured by the assessment tools employed, and so warrant further more systematic investigation.

Symptoms of urinary dysfunction such as urge, frequency, retention and incontinence are recorded as a formal urological problem in more than 20% of patients in this study (see

Table **8**). These symptoms are common and troublesome, but potentially treatable. In an allied study on a subset of these patients employing more specific questionnaires, 83% of patients reported lower urinary tract symptoms. In the same study, 64% also reported bowel symptoms and 83% sexual symptoms (Lad, Parkinson, *et al.,*manuscript in preparation). The present study recorded 14.4% as having symptoms of gastritis, acid reflux or hiatus hernia. This was far commoner than chronic constipation (6.6%) or diarrhoea (1.2%) and may relate in part to posture. These symptoms warrant greater clinical recognition because of their treatability and effect on quality of life.

Patients with FRDA often describe cold, discoloured feet. In an exploratory study of 50 consecutive patients toward the end of the project, 41 (82%) complained of persistently and unpleasantly cold feet. Twenty-five (50%) described colour changes, including 9 (18%) white, 8 (16%) red, 10 (20%) blue, 15 (30%) purple and 5 (10%) mottled. The underlying cause of these abnormalities is not clear. Resting blood pressure is frequently low in FRDA and syncope in response to phlebotomy is not uncommon. Of the 164 patients who had their blood pressure measured, 30 (18.3%) had systolic blood pressure below 100mmHg and 87 (53.0%) below 120mmHg, with the lowest pressure measured of 66mmHg. Of course, this is predominantly a young cohort which would not be expected to have a significant degree of hypertension. Autonomic dysfunction is not well studied in FRDA and there may well also be loss of postural vascular reflexes in response to immobility. However, anecdotally, this is a common and unpleasant problem, and one which is difficult to treat.

Figure 36 summarizes the common clinical features of FRDA as recorded in the EFACTS Registry (scoliosis, visual impairment, pes cavus, cardiomyopathy, hearing impairment, dysuria, gastritic symptoms, diabetes), the INAS (dysarthria, dysphagia) and the SNE (talipes equinus, talipes varus). Figure 37 summarizes the common examination findings found in FRDA as recorded in the SDFS (wheelchair use), the INAS (areflexia, paresis, plantars, spasticity, amyotrophy, hyperreflexia) and the SNE (vibrational, proprioceptive and pin prick sensory loss).

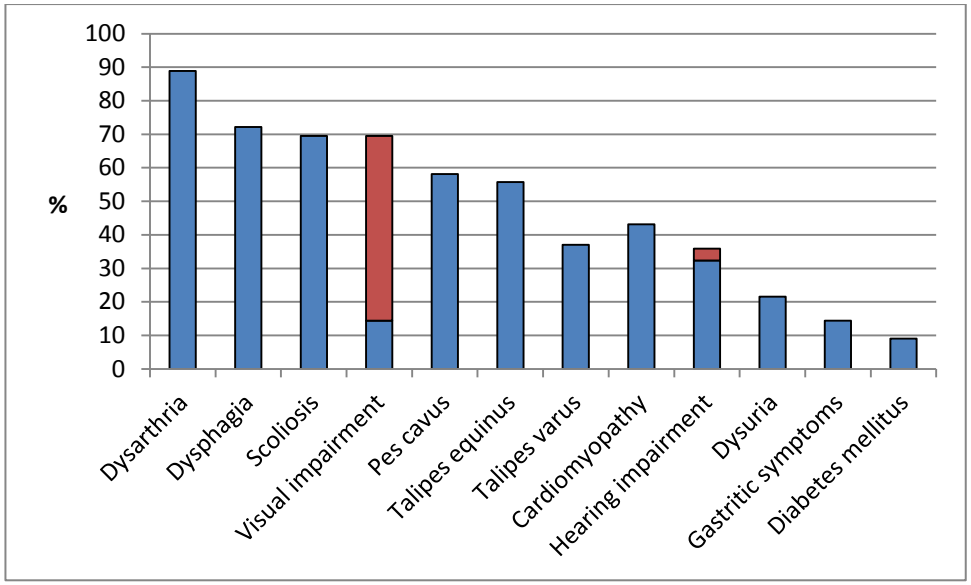


Figure 36: Common clinical features of FRDA.
Blue section indicates proportion likely to arise directly from FRDA. For explanation, see Section 2.3.5 above.

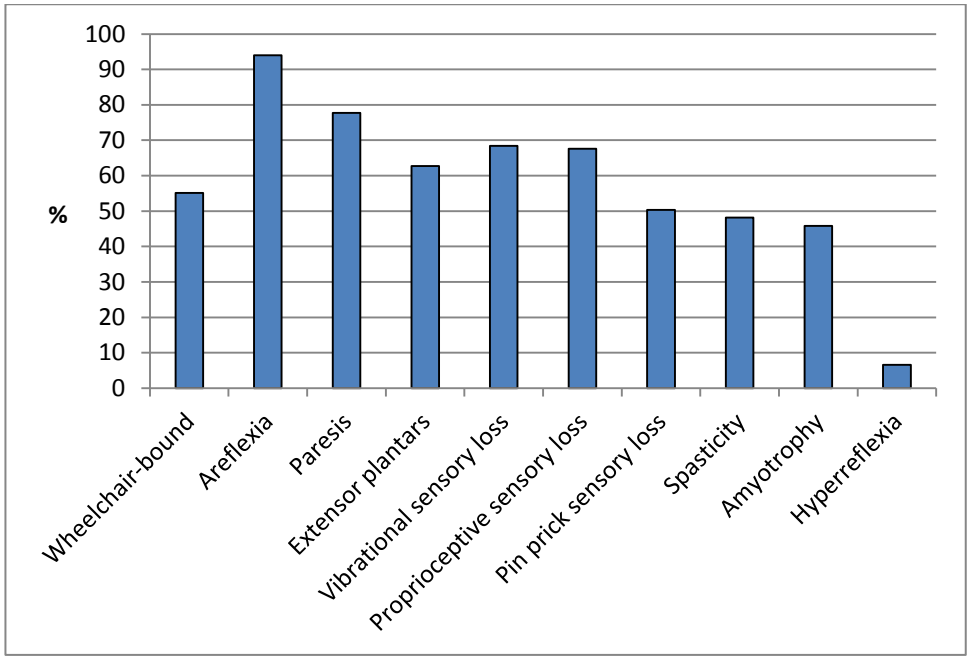


Figure 37: Common examination findings in FRDA

Thus, this study constitutes the largest ever natural history study of FRDA in the UK and adds considerable deep phenotyping data to our knowledge of this condition which may be used both in the clinical and research settings. Possible criticisms of the study include the lack of imaging data, although in fact, once the diagnosis is made, this rarely forms an important part of the assessment. Another criticism is the lack of cognitive assessment in the study. In fact, the original EFACTS battery of tests included

a single neuropsychological test, involving an assessment of phonemic verbal fluency (data not presented). There were problems in comparing the results between different European centres. It has been decided to use the Montreal Cognitive Assessment (MoCA) in future assessments although this also has problems as many participants cannot complete the visuoconstructive portions of the task which involve drawing a cube and a clockface, as well as the trailmaking test, because of poor manual dexterity rather than cognitive impairment. The study has no control group with which to compare. This feature is currently being addressed but data were not available at the time of data analysis.

The data allow the investigation of a number of features, such as the nature of different phenotypes based on age at onset, the phenomenon of growth retardation in young-onset patients, correlations between clinical and genetic markers, and the validity of different rating scales. These will be discussed below.

2.4.2 Clinical Characterization : Classical and Atypical Phenotypes

The classical presentation of FRDA as defined by Harding (1984) involved symptom onset before the age of 25, progressive ataxia, absent lower limb reflexes and extensor plantar responses, amongst other features. The discovery of the causative mutation in 1996 enabled the identification of atypical cases, notably those with late-onset, retained reflexes, pyramidal signs and a relative paucity of non-neurological features such as cardiomyopathy and diabetes. Little attention has been paid in the literature to those patients with early-onset disease (below the age of 5 years) who typically have a much more severe phenotype. We wished to investigate this matter further to see if these groups represented distinct nosological entities.

Table 24A: Comparison of GAA1 size, total ADL, total SARA, INAS count and SDFS for early-onset, classical and late-onset subtypes of FRDA in EFACTS

Feature	Early-onset (≤5 years)[a]	Classical (6-24 years)[b]	Late-onset (≥25 years)[c]	ANOVA F value (p value)
Age at onset	3.0±1.4 (p<0.0005)	12.5±4.5 (p<0.0005)	35.4±9.7 (p<0.0005)	230.1 (p<0.0005)*
Age at exam	25.5±7.4 (p=0.008)	33.5±12.6 (p<0.0005)	49.9±8.6 (p<0.0005)	23.9 (p<0.0005)*
Disease duration	22.5±7.4 (p=1.000)	21.0±12.1 (p=0.054)	14.5±6.7 (p=0.065)	3.3 (p=0.04)
GAA1	759.7±197.6[d,m] (p=0.655)	695.8±221.3[e,n] (p<0.0005)	290.5±208.8[f] (p<0.0005)	20.3 (p<0.0005)*
Total ADL	19.3±6.6[d] (p=0.152)	15.7±8.5[g] (p=0.0005)	8.4±4.4[h] (p<0.0005)	9.8 (p<0.0005)*
Total SARA	26.9±7.9[d] (p=0.273)	32.2±9.9[i] (p<0.0005)	12.4±8.0[h] (p<0.0005)	13.1 (p<0.0005)*
INAS count	5.2±1.6	5.1±1.7[j]	4.3±1.2[k]	2.6 (p=0.079)
SDFS	5.5±1.0[l] (p=0.343)	5.0±1.4[e] (p=0.05)	3.9±1.4[h] (p=0.0005)	7.4 (p=0.0005)*

Values are for mean ± SD in first 3 columns
p values quoted in first 3 columns are for *post-hoc* analysis with Bonferroni corrections pre-adjusted in SPSS (ie significant at 0.05)
Column 1 compares early-onset with classical. Column 2 compares classical with late-onset. Column 3 compares late-onset with early-onset.
[a]*n*=23; [b]*n*=125; [c]*n*=19; unless otherwise specified below:
[d]*n*=22; [e]*n*=114; [f]*n*=17; [g]*n*=122; [h]*n*=18; [i]*n*=120; [j]*n*=124; [k]*n*=19; [l]*n*=21
[m]excludes 1 compound heterozygote with macrodeletion
[n]exlcudes 7 compound heterozygotes with point mutations
*Significant at Bonferroni-adjusted p value of 0.006 (ie 0.05/8)

Table 24B: Comparison of total ADL, total SARA, INAS count & SDFS for early-onset, classical and late-onset subtypes of FRDA in EFACTS corrected for disease duration

Feature	Early-onset (≤5 years)[a]	Classical (6-24 years)[b]	Late-onset (≥25 years)[c]	MANCOVA F value (p value)
Disease duration[d]	22.5±7.4	21.0±12.1	14.5±6.7	-
Total ADL[e]	18.4±1.3 (p=0.18)	15.7±0.6 (p=0.012)	11.0±1.5 (p=0.001)	6.9 (p=0.001)*
Total SARA[e]	25.9±1.7 (p=0.298)	22.9±0.7 (p<0.0005)	15.2±1.9 (p<0.0005)	9.9 (p<0.0005)*
INAS count[e]	5.1±0.3 (p=1)	5.1±0.1 (p=0.488)	4.6±0.3 (p=0.727)	1.0 (p=0.363)
SDFS[e]	5.4±0.2 (p=0.42)	5.0±0.1 (p=0.022)	4.2±0.3 (p=0.005)	75.4 (p=0.005)*

[a]*n*=22; [b]*n*=119; [c]*n*=18 (cases for which all values available allowing MANCOVA)
[d]mean±SD
[e]mean±SEM; covariates are evaluated at disease duration=20.34 years
p values quoted in 1st 3 columns are for *post-hoc* analysis with Bonferroni corrections pre-adjusted in SPSS (ie significant at 0.05)
*Significant at Bonferroni-adjusted p value of 0.0125

Table 25: Comparison of examination findings from INAS and EFACTS assessment for early-onset, classical and late-onset subtypes of FRDA patients from EFACTS

Feature	Early-onset (≤5 years)	Classical (6-24 years)	Late-onset (≥25 years)	χ^2 Value[c] (p value)	Corrected χ^2 Value[d] (p value)
Gender	9:14	56:69	7:1	0.852 (p=0.653)	-
Amyotrophy[a]	15/23 (65.2)	58/124 (46.8)	3/19 (15.8)	10.4 (p=0.005)	1.7 (p=0.194)
Paresis[a]	18/23 (78.3)	102/124 (82.3)	9/19 (47.4)	11.6 (p=0.003)*	0.1 (p=0.807)
Areflexia[a]	23/23 (100)	123/124 (99.2)	10/19 (52.6)	64.8 (p<0.0005)*	8.0 (p=0.005)
Hyperreflexia[a]	0/23 (0)	4/124 (3.2)	7/19 (36.8)	32.0 (p<0.0005)*	0.3 (p=0.565)
Sensory loss[a]	19/24 (79.2)	102/122 (83.6)	15/18 (83.3)	6.7 (p=0.352)	2.8 (p=0.092)
Spasticity[a]	12/23 (52.2)	56/124 (45.2)	12/19 (15.0)	2.3 (p=0.316)	0.2 (p=0.681)
Extensor plantars[a]	14/23 (60.9)	81/124 (65.3)	9/19 (47.4)	2.3 (p=0.316)	1.1 (p=0.292)
Dysarthria[a]	20/22 (90.9)	108/122 (88.5)	16/18 (88.9)	0.1 (p=0.948)	3.1 (p=0.077)
Dysphagia[a]	17/22 (77.3)	88/122 (72.1)	12/18 (66.7)	0.6 (p=0.757)	0.3 (p=0.565)
Wheelchair-bound[b]	15/21 (71.4)	66/114 (57.9)	2/18 (11.1)	16.6 (p<0.0005)*	0.6 (p=0.447)
Cardio-myopathy[b]	16/23 (69.6)	54/121 (44.6)	2/18 (11.1)	14.0 (p=0.001)*	7.6 (p=0.006)
Diabetes mellitus[b]	3/21 (14.3)	10/114 (8.8)	0/18 (0)	2.6 (p=0.274)	0.5 (p=0.489)
Pes cavus[b]	18/23 (78.3)	74/124 (59.7)	4/19 (21.1)	14.6 (p=0.001)*	4.7 (p=0.031)
Scoliosis[b]	21/23 (91.3)	86/124 (69.4)	9/19 (47.4)	9.6 (p=0.008)	4.1 (p=0.042)

Values are for n/N (%)

[a]Categorical values (presence or absence) taken from INAS count calculation. A categorical value was calculated for dysarthria and dysphagia from the INAS data. Sensory loss refers to vibrational sensory loss at the external malleolus from the INAS

[b]Categorical values (presence or absence) taken from EFACTS Registry

[c]Uncorrected Pearson χ^2 test

[d]Wald χ^2 test corrected for disease duration using ordinal logistic regression

*Significant at Bonferroni-adjusted p value of 0.003 (ie 0.05/15)

Table 24A shows the age at onset, age at examination, disease duration, GAA1 size, total ADL, total SARA, INAS count and SDFS for the 167 FRDA patients in the EFACTS

study for which data were available at the baseline assessment divided according to age at onset (early-onset ≤ 5 years, classical 6-24 years, late-onset ≥ 25 years). Column 4 shows the ANOVA F-values and p-values. After Bonferroni adjustment for multiple testing the significance level is p<0.006. Table 24B shows total ADL, total SARA, INAS count and SDFS for these three groups corrected for disease duration as a confounding factor using a multivariate analysis of covariance (MANCOVA).

In the uncorrected analysis (table 24A), the mean age at onset was significantly different between the three groups as this was the criterion on which the groups were selected. The mean age at examination was significantly different between the three groups but the difference in disease duration did not survive Bonferroni correction. The means of GAA1 size, total ADL, total SARA and SDFS were significantly different between the three groups and these parameters went on to individual pairwise *post-hoc* testing for which p values pre-adjusted by SPSS are quoted (significant at p<0.05). The GAA sizes for the eight compound heterozygotes were excluded from all calculations involving GAA size, although these patients were included in the analysis of their clinical features. Figure 38 shows the mean ± SD for GAA1 size, total ADL, total SARA, INAS count and SDFS divided by subtype. Significant differences were found between classical and late-onset subtype, and between early- and late-onset subtype, but not between early-onset and classical subtype for GAA1 size, total ADL, total SARA and SDFS, with all values increasing with later onset of disease.

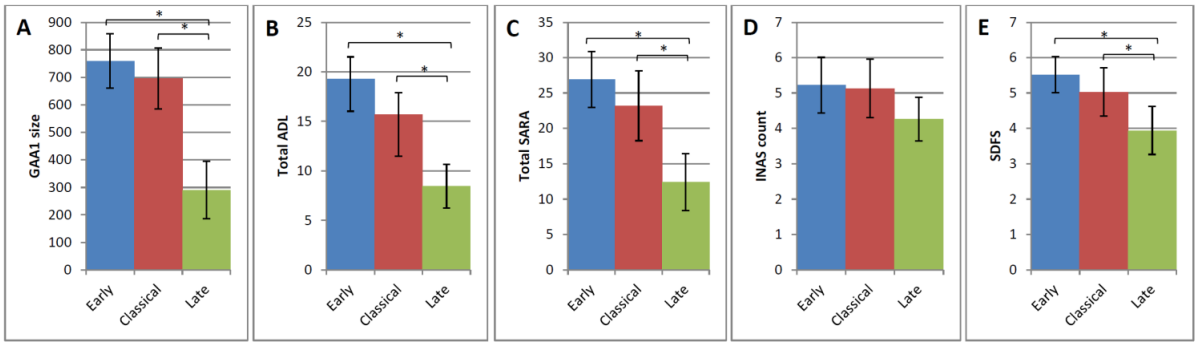


Figure 38: Comparison of (A) GAA1 size, (B) total ADL, (C) total SARA, (D) INAS count and (E) SDFS for early-onset, classical and late-onset subtypes of FRDA from EFACTS.
Error bars=SD. *Significant difference at p<0.05 after Bonferroni adjustment

After correction for differences in disease duration between the three groups (Table 24B), the results remain broadly similar with statistically significant differences in the

total ADL, total SARA and SDFS but not the INAS count, underlining its inferiority as an assessment tool in FRDA. *Post-hoc* analysis shows that all the significant differences after correction are between either (a) the early-onset and late-onset groups; or (b) the classical and late-onset groups. There were no statistically significant differences between the early-onset and classical groups, suggesting that these are part of a continuum rather than distinct nosological subtypes.

Table 25 shows proportion of patients with various examination findings and associated features derived from the INAS data and EFACTS assessment for which data were available at baseline divided according to age at onset subtype. These are binary categorical data showing either presence or absence of the symptom or feature and do not express its severity. Column 4 shows the uncorrected Pearson χ^2 and p-values. After Bonferroni adjustment for multiple testing the significance level is $p<0.003$. Column 5 shows the Wald χ^2 and p-values corrected for disease duration as a confounder.

There were no differences in gender between the three groups. There were significant differences between the three subtypes for muscle weakness, areflexia, hyperreflexia, cardiomyopathy, pes cavus and wheelchair-bound status. The late-onset group had significantly less muscle weakness and over 50% had retained reflexes. Hyperreflexia was only really seen in the late-onset group. A very small proportion of the late-onset group were wheelchair-bound or had cardiomyopathy (about 10% in each case). Pes cavus was similarly less prominent in the late-onset group. Differences in muscle wasting and scoliosis did not survive Bonferroni correction although in each case their prevalence decreased with later onset. There were no significant difference in dysarthria, dysphagia, vibrational sensory loss or the presence of extensor plantar reflexes between the three groups which were all very common throughout. Surprisingly, spasticity appeared to decrease with later onset although there was no significant difference between the groups. There was also no significant difference in diabetes although there were no cases amongst the late-onset group, and the early-onset group had the highest prevalence, although at a low level (14%). Figure 39 and Figure 40 show these features graphically. After correction for disease duration, there were significant differences in areflexia ($p=0.005$), cardiomyopathy ($p=0.006$), pes

cavus (p=0.031) & scoliosis (p=0.042) but none of these survived Bonferroni correction for multiple tests (Bonferroni significance level 0.05/14=0.0036).

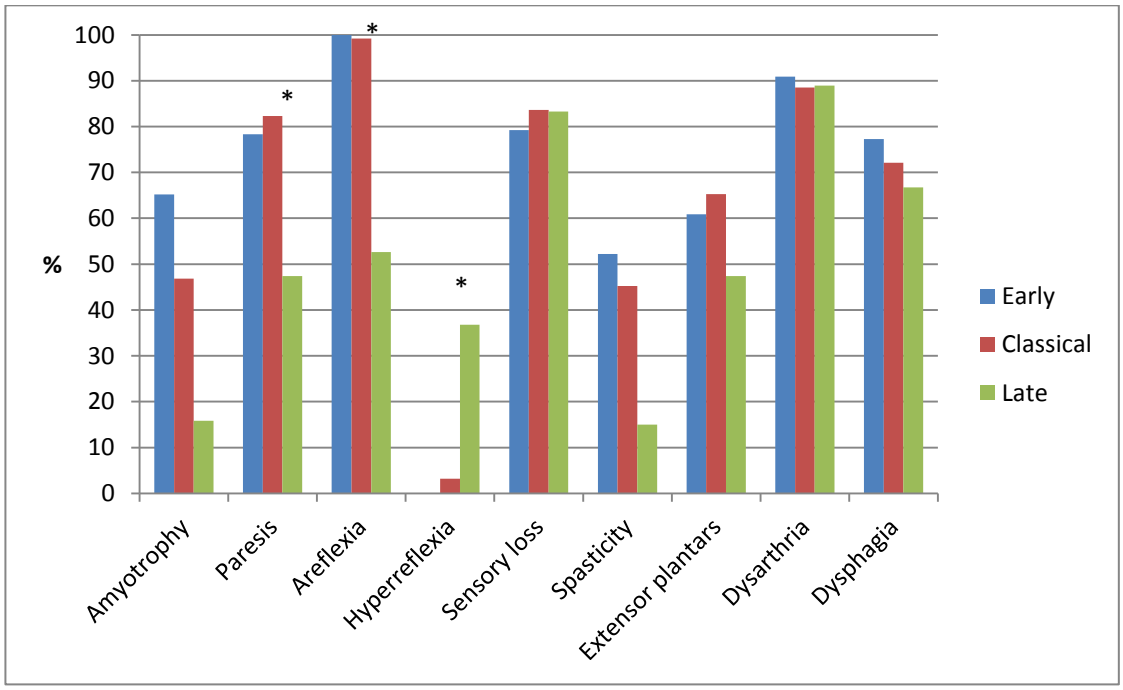


Figure 39: Comparison of examination findings from INAS for early-onset, classical and late-onset subtypes of FRDA patients in EFACTS.
*Differences significant at p=0.05 after Bonferroni correction

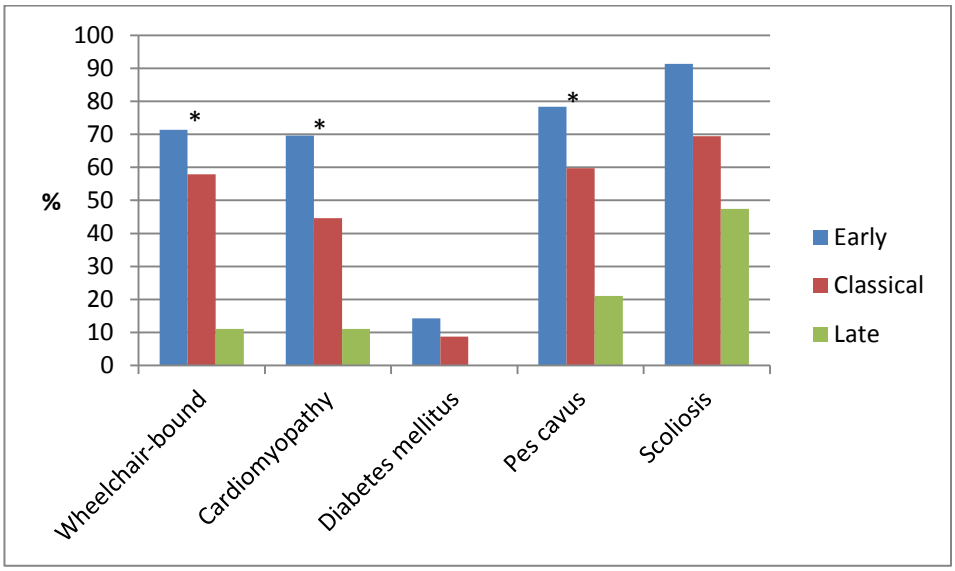


Figure 40: Comparison of examination findings from EFACTS assessment for early-onset, classical and late-onset subtypes of FRDA patients in EFACTS.
*Differences significant at p=0.05 after Bonferroni correction

Taken together, these data suggest that early-onset, classical and late-onset FRDA are not distinct nosological entities, and indeed the distinguishing feature chosen for

analysis was essentially arbitrary, although in the case of the late-onset subtype, on the basis of previously published diagnostic criteria (Harding 1984) and descriptions of atypical forms such as late-onset FRDA (LOFA) and FRDA with retained reflexes (FARR) (De Michele *et al.* 1994, Klockgether *et al.* 1996). Rather, FRDA forms a continuum in which there is a progressively more severe phenotype with earlier age at onset. This is particularly marked by increasing weakness, immobility and associated features of cardiomyopathy, pes cavus and areflexia. Muscle wasting, scoliosis and diabetes are probably also more prominent. There is greater justification for distinguishing LOFA from the other groups and this does appear to have some characteristic features of retained and in some cases brisk reflexes, together with an absence of diabetes and very low prevalence of cardiomyopathy, pes cavus and wheelchair confinement. Section 2.4.4 below explores the correlation between some of these features.

2.4.3 Growth Retardation

As noted by Harding (Harding 1984), patients with FRDA with onset of symptoms in childhood frequently do not develop muscle bulk and can appear to have growth retardation. The present study affords some scope to investigate this observation.

World Health Organization (WHO) growth charts extend up to age 20 (http://www.who.int/growthref/en/). The Royal College of Paediatrics and Child Health (RCPCH) uses the same data for their growth charts for children in the UK above the age of 4 (http://www.rcpch.ac.uk/growthcharts). There were 24 patients in the study between the ages of 16 and 20 (8 male, 16 female). Their heights and centile for age and sex are shown in Table 26 and plotted on the appropriate WHO growth chart in Figure 41. The mean height for the male participants corresponded to the 43.3th centile (range 30.9-65.4). The mean height for the female participants corresponded to the 27.0th centile, although one value was well below normal. Even excluding this value, the mean female height centile was 32.7. The mean height centile for all patients in the study aged 16-20 was 32.4.

These data seem to add some credence to the idea of growth retardation amongst FRDA patients. If this were the case, one might expect a correlation between age at

onset and height as the earlier the age at onset, the greater the growth retardation. Figure 42 shows the height of the 102 patients of 40 or less at the time of baseline assessment plotted against age at onset. There is no correlation between the two parameters (Spearman's rank correlation coefficient R=-0.113, R^2=0.013, p=0.259).

Only a small number of participants in the study were appropriate for the first calculation, and as Figure 41 shows, they appear on the portion of the graph which has plateaued, as the patients are adults. Indeed, not all growth charts extend to age 20. It would be interesting to repeat this calculation for children and in a larger sample size and with age-matched control data.

Table 26: Height & centile for EFACTS patients aged 16-20 (based on WHO growth chart)

Sex	Age	Age at onset	Height (cm)	Centile
M	16	2	175	54.2
M	17	8	175	48.1
M	18	6	170	30.9
M	18	4.5	178	52.1
M	18	1	183	65.4
M	19	7	165	16.9
M	19	14	177	48.9
M	20	8	170	30.3
F	16	3	153	18.7
F	17	10	155	24.1
F	17	12	164	51.4
F	18	12	151	11.1
F	18	11	167	60.1
F	18	12	159	35.6
F	19	10	165	54.0
F	19	1	170	69.3
F	19	7	128	-59.3
F	19	4	152	14.2
F	19	8	152	14.2
F	19	12	148	1.9
F	19	6	161	41.7
F	19	10	160	38.7
F	20	17	159	35.6
F	20	10	154	20.3

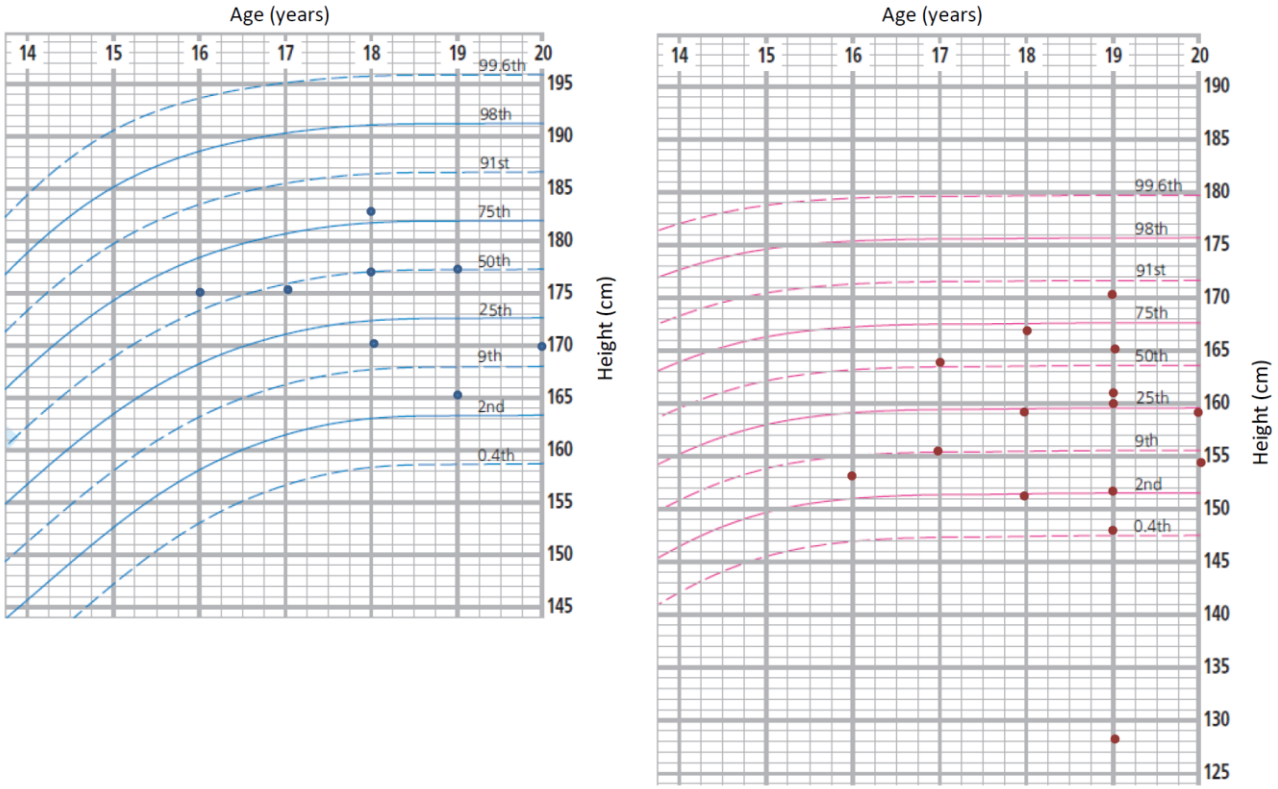


Figure 41: Height of EFACTS patients aged 16-20 plotted on WHO growth charts. Male (left), female (right).

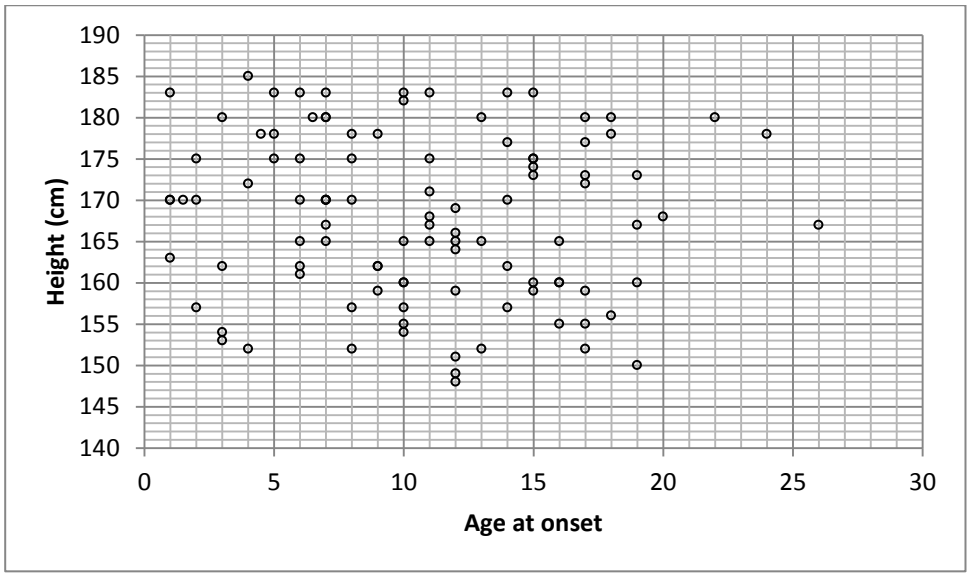


Figure 42: Height of EFACTS patients of 40 or less plotted against age at onset

2.4.4 Correlation with GAA Size and Clinical Rating Scales

Previous studies have shown an inverse correlation between the size of the shorter GAA expansion in the frataxin gene and various markers of disease severity including

age at onset and time to wheelchair use (Dürr et al. 1996). GAA expansion size inversely correlates with frataxin protein level and mRNA expression in peripheral blood cells (Saccà *et al.* 2011). Only GAA expansion size was available at the time of data analysis. Figure 43 shows the negative correlation between the size of the shorter GAA expansion (GAA1) and age at symptom onset. This is greater than the correlation between the longer GAA expansion (GAA2) and age at onset. This may be because the GAA1 size is typically spread over a greater range of values than is GAA2 size, as shown in Figure 10 and discussed in the text at 2.3.3, particularly for shorter expansions. Almost all the GAA2 sizes are greater than 500 repeats which are associated with very low levels of frataxin production. Thus, there is likely to be less difference in frataxin levels between these larger expansions, and hence the GAA2 size exerts less influence over the clinical phenotype.

Figure 44 shows the positive correlation between GAA1 size and various clinical assessment tools recorded in the study, of which the total SARA score shows the strongest correlation, although clearly with a wide spread of values. Figure 45 shows the positive correlation between age at onset and the same range of clinical assessment tools, again showing the strongest correlation with the total SARA score and overall a far stronger correlation than with GAA1 size. Figure 46 shows the positive correlation between disease duration and the four clinical assessment tools, this time showing the strongest correlation with the total ADL. This is the strongest correlation found. This also gives an impression of disease progression based on cross-sectional rather than longitudinal data. Values shown are for Pearson's correlation. This is the first study to correlate GAA expansion size with these clinical rating scales.

Taken together, these findings suggest that the SARA and the ADL are the best markers of clinical severity, and disease duration is the strongest predictor of disease progression. Thus, for every additional 100 GAA1 repeats, disease onset is predicted to be 2.1 years earlier, so that a GAA1 size of 100 predicts an age at onset of 25.7 years, and a GAA1 size of 1000 predicts an age at onset of 3.7 years. Many diagnostic labs use a GAA size of 100 as the lower limit of a pathological expansion. It is interesting that the Harding diagnostic criteria formulated before the *FXN* gene had been discovered, stipulated that disease onset should be below the age of 25 (Harding 1981) which

matches exactly with this result. Every additional 100 GAA1 repeats increase the SARA scale by 1.2 points on average, and the ADL by 0.7 points. Both early age at onset and disease duration predict clinical severity, so that every 10 years earlier that disease commences contributes 5.1 points on the SARA and 3.8 points on the ADL, and every additional 10 years of disease duration adds 5.4 points on the SARA and 4.8 points on the ADL.

Samples have already been collected for assessment of frataxin mRNA and protein levels. At present the greatest correlation between a clinical and an underlying pathological marker is between age at onset and GAA1 size. However, this still only explains 33.1% of the variation [see Figure 43 (A)]. Alternative explanations may include epigenetic factors acting on the *FXN* gene. Downstream markers of gene function may therefore provide a more accurate prediction of clinical severity.

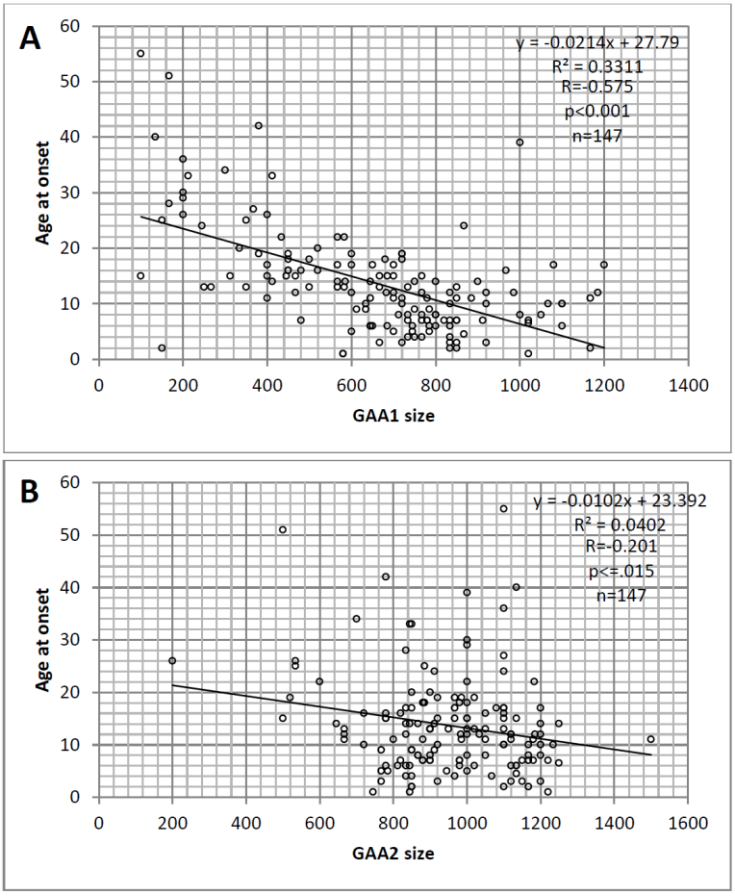


Figure 43: Correlation between age at onset and (A) GAA1 size (B) GAA2 size for EFACTS patients

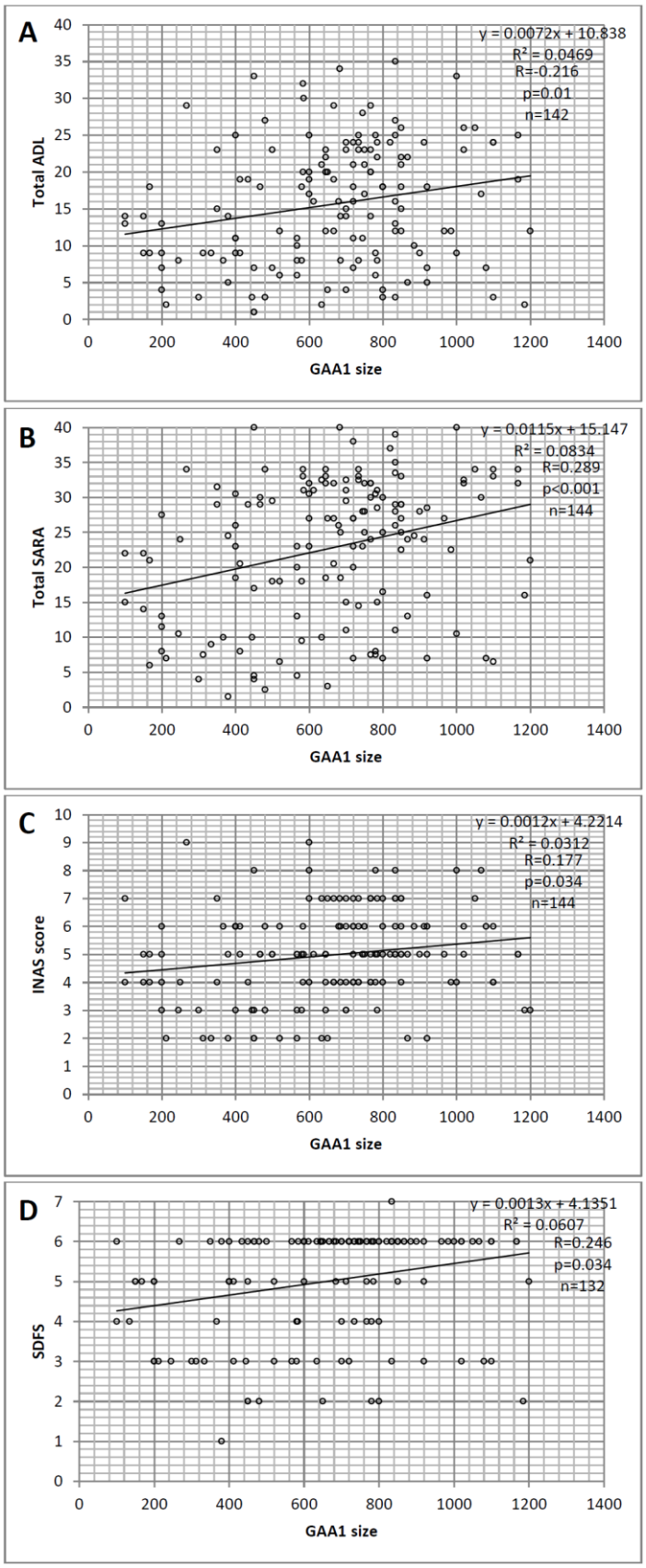


Figure 44: Correlation between GAA1 size and (A) total ADL (B) total SARA (C) INAS score (D) SDFS for EFACTS patients

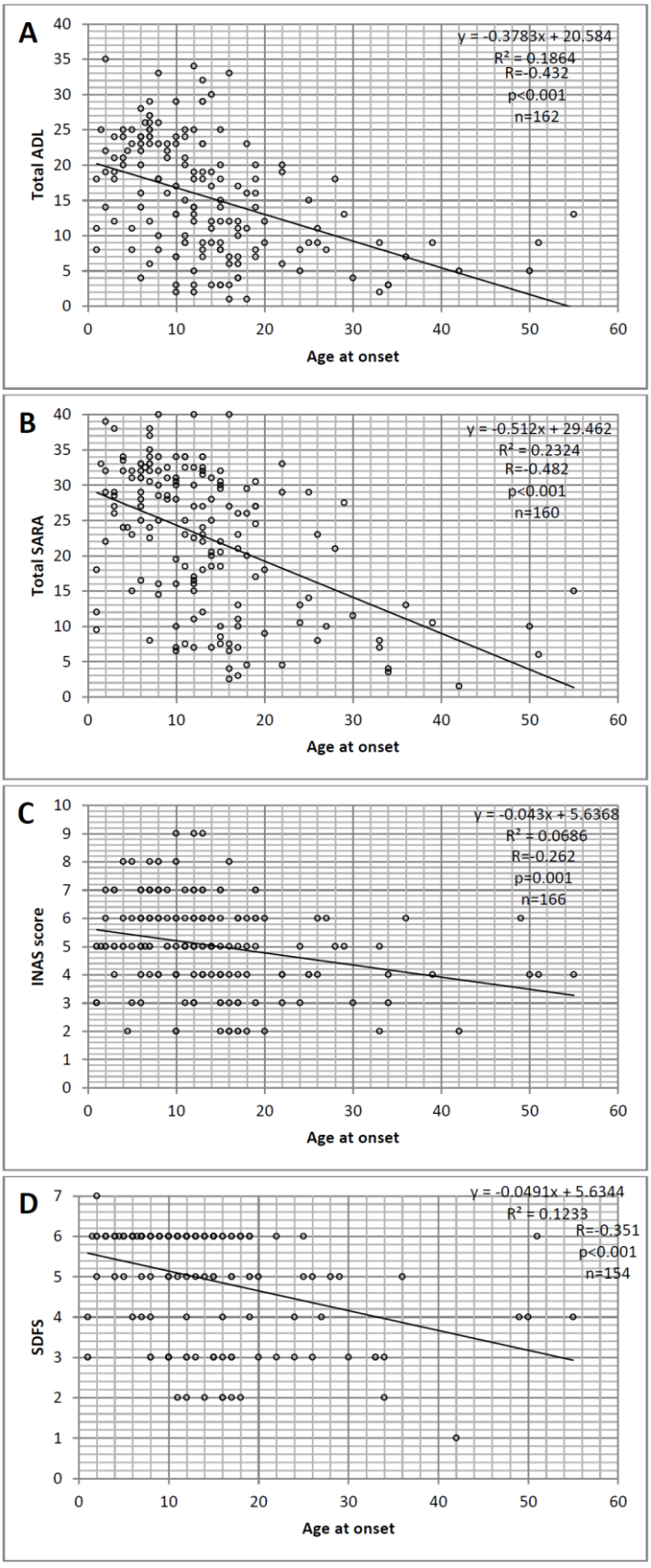


Figure 45: Correlation between age at onset and (A) total ADL (B) total SARA (C) INAS score (D) SDFS for EFACTS patients

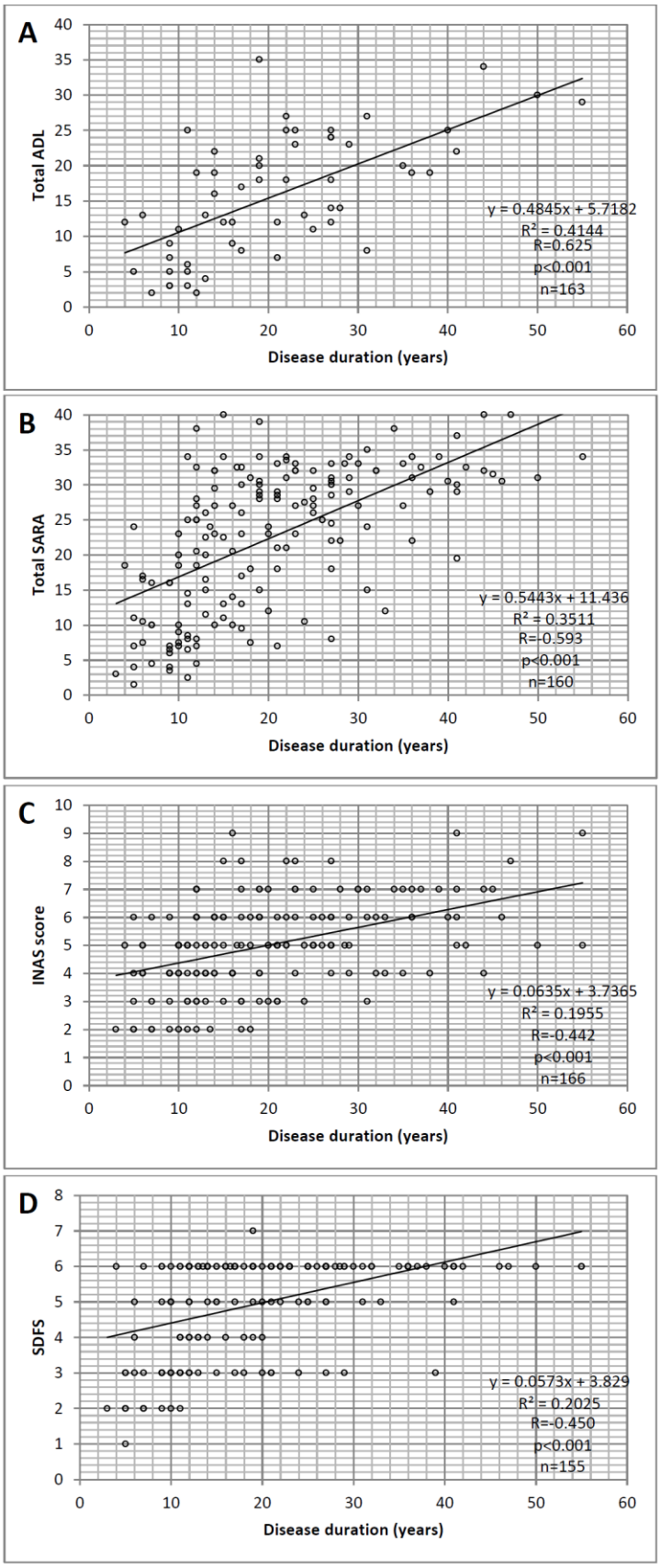


Figure 46: Correlation between disease duration and (A) total ADL (B) total SARA (C) INAS score (D) SDFS for EFACTS patients

2.4.5 Validity of Clinical Rating Scales

One of the principal objectives of EFACTS was to validate a variety of clinical rating scales and systematically collect a mass of baseline and longitudinal data specifically related to FRDA in order that they might subsequently be used as part of appropriately planned and powered interventional trials. To that end, four scales were used, *viz*. the SARA, INAS, SCAFI and CCFS. The data relating to the CCFS are being analysed by a collaborator (Pr. Alexandra Dürr, Hôpital de la Pitié-Salpetrière, Paris) and are not presented in this thesis. In addition, the ADL questionnaire from the FARS and the SDFS were administered. These scales were supplemented by data collected as part of the EFACTS assessment and in the London centre by the SNE. None of the scales has previously been used in such a large and clinically well characterized sample as the EFACTS cohort.

The ADL questionnaire was taken from the FARS (Subramony et al. 2005) and has previously been studied in FRDA (Lynch et al. 2006, Friedman et al. 2010, Bürk et al. 2009). It is quick to administer and provides a rounded assessment of the burden of disease in FRDA including bladder function which this thesis has shown is a significant and probably undervalued feature of the condition. It takes into account practical functions such as washing, dressing and use of cutlery, which arguably are more meaningful for patients, if perhaps less objective than many examination findings. Each category is divided into a simple semi-quantitative 5-point scale, but each point is precisely defined preventing inter-rater variation : for example, the use of cutlery category uses such descriptive phrases as ‘mildly slowed and clumsy but no assistance required’, ‘can cut most dishes but slowed and clumsy, some assistance required’ and ‘dishes have to be cut by caregiver, can eat slowly’ rather than less precise terms such as ‘mild’, ‘moderate’ and ‘severe’.

It was initially envisaged that the questionnaire would be sent to patients and completed in advance of the clinical assessment. However, the graphic nature of some of the descriptions caused distress to some patients such as ‘helpless’, ‘completely depending on help’ and ‘incapable of walking even with support’ and so it was decided that the examiner would administer the questionnaire in clinic. This may have

engendered the additional benefit of improving inter-rater variability as only two clinicians saw patients as part of the project and the vast majority were seen by the author. However, it did represent a deviation from protocol. If the ADL questionnaire is to be issued to patients to complete themselves, it perhaps should contain more sympathetically worded responses. Figure 15 shows that, of the contributory categories, use of cutlery, dressing, washing and sitting show an even spread of values. Speech and swallowing are skewed toward the lower values but with a good spread of values up to the middle range. Only the walking and falls categories show the greatest proportion in the highest score, raising the possibility of a ceiling effect in these categories. Overall, when all the values are pooled, there is a very good spread of values across the five summed categories of the ADL. The total ADL score correlates with GAA1 size, age at onset and disease duration (see Section 2.4.4 above).

The SARA was initially described for use in the SCAs (Schmitz-Hübsch et al. 2006) but has subsequently been studied in a variety of ataxias including FRDA (Weyer et al. 2007, Bürk et al. 2009). It is quick and easy to administer, and assesses a broad range of functions known to be affected in FRDA including speech, gait, and measures of upper and lower limb ataxia. It employs a variety of examination techniques familiar to many clinicians, such as the 'nose-finger test', 'heel-shin test' and the test of fast alternating hand movements traditionally used to assess dysdiadocokinesis. The scores are precisely defined to reduce inter-rater variability : for example, in the 'finger chase test' the degree of dysmetria is 'none', '<5cm', '<15cm', '>15cm' or unable to perform test. Whilst being precise and objective, the scale solely assess ataxia which is not the only cause of disability in FRDA, and only utilizes clinical examination findings. Thus, it may not be functionally meaningful for a patient with FRDA who is wheelchair-bound to be informed that the heel-shin test has progressed from three times in three cycles to four or more times in three cycles.

Potentially more problematical for clinical trials is that it is heavily weighted toward lower limb function. Figure 16 shows that the vast majority of patients already occupy the highest score within the gait, stance, sitting and heel-shin test categories when first assessed at their baseline visit. This is consistent with the knowledge that more than half of the cohort were wheelchair-bound at this assessment. This introduces a

potential ceiling effect into the scale which might prompt researchers to exclude wheelchair-bound patients from clinical trials in order to improve sensitivity to change, with the possible effect that subsequent commissioning groups and prescribers might exclude these patients from treatment. The speech, finger chase, nose-finger test and fast alternating hand movement categories are much more evenly spread with modal values in the middle of each category. It is not possible to pool individual scores in the same way as for the ADL as each of the categories has a different number of scores. However, the total SARA score does still correlate with GAA1 size, age at onset and disease duration (see Section 2.4.4 above).

The INAS was first described in SCA 1,2,3 & 6 in order to assess non-ataxic symptoms which may complicate these primarily ataxic disorders (Jacobi *et al*. 2013). It was found to have good reproducibility but unsatisfactory responsiveness. The authors concluded that it was a valid measure of extracerebellar involvement in these conditions but should not be used as a primary outcome measure. It has not hitherto been validated in FRDA. The INAS assesses 34 separate categories both of clinical signs elicited by the examiner and symptoms reported by the patient. Most are assessed on a semi-quantitative scale of zero to three (none, mild, moderate, severe) without further precise definition leading to these assessment being open to some inter-rate variation. The only fully objective sign is the assessment of vibrational sensibility using the Rydel-Seiffer graduated tuning fork, although four of the symptom categories are precisely defined (episodic vertigo, speech problems, handwriting problems, spontaneous cramps). The scale is necessarily more time-consuming to administer than the ADL and SARA and requires considerable experience of neurological assessment as it essentially involves a full neurological examination with the recognition of a variety of movement disorders and variations in muscle tone (*eg* spasticity versus rigidity versus dystonia). The assessment of eye movements is particularly rigorous.

The INAS collects a large amount of useful examination information (see Table 14), the extracerebellar features of which are extracted using a slightly complicated technique to give the 'INAS count' covering 16 domains (see Table 13 and Figure 17). The INAS count domains highlight those features which are prominent in FRDA (areflexia, vibrational sensory loss, paresis, extensor plantar responses, muscle atrophy, urinary

dysfunction, brainstem oculomotor signs, and perhaps surprisingly, spasticity). However, it includes many domains which are rarely affected in FRDA (such as hyperreflexia and cognitive impairment) and domains which are practically never affected (such as myoclonus, chorea and parkinsonism). Nevertheless, the INAS score does correlate with GAA1 size, age at onset and disease duration, albeit with considerably lower correlation coefficients than for the ADL and SARA. The absence of so many unaffected domains means than the maximum score obtained by any patient was 9 out of 16, meaning that the score contains a considerable floor effect in these domains.

As stated, the INAS collects a large general amount of examination information which is not used in the calculation of the final INAS count. This information added considerably to the broad clinical description of FRDA given in Section 2.4.1 above and groups the signs so that they are easier to analyze and present than, for example, in the SNE. The INAS, for example, reveals the prominence of broken smooth pursuits, square wave jerks and saccadic dysmetria over other oculomotor abnormalities (see Figure 21).

The SCAFI is the functional score taken from the FARS (Subramony et al. 2005) and has previously been studied in FRDA (Lynch et al. 2006, Bürk et al. 2009, Friedman et al. 2010). The SCAFI assesses three functions : it assesses mobility by means of an 8-metre timed walk (8mTW), manual dexterity by means of a 9-hole peg test (9hPT) and speech by means of the 'pa-ta test'. As more than half of the patients in the cohort were wheelchair-bound, this meant that more than half could not complete the task in its entirety. These patients are all assigned the same high score by default, leading to an immense ceiling effect in this scale. Indeed, only 31.7% of patients in the cohort completed the 8mTW as many, who are able to mobilize at home with a walking aid, had not brought this to clinic. If patients are assessed in their own homes, there is often not a suitable flat furniture-free 8-metre course over which to assess the patient. A considerable proportion (around 30%) also cannot complete the 9hPT because of severe lack of manual dexterity, and many find the four replications of the task tiring and distressing. The scale also requires specific equipment (the Rolyan plastic one-piece 9hPT apparatus) which is not readily available in most clinics. Figure 22 shows

that the raw data for the 8mTW and 9hPT for those patients who are able to undertake these tasks are heavily skewed toward lower values. Only the pa-ta test is normally distributed, able to be completed by a high proportion of patients and does not require special equipment.

Thus, the ADL provides a reliable symptom-based assessment and the SARA a reliable examination-based assessment of the extent of disease in FRDA. The INAS provides a large amount of useful information but the INAS count is less relevant to FRDA. The majority of FRDA patients are unable to complete the SCAFI making it less reliable in FRDA.

2.4.6 Disease Progression

The EFACTS project enables the study of clinical rating scales both cross-sectionally as above, but also longitudinally which has only rarely been undertaken before (Fahey *et al.* 2007, Friedman *et al.* 2010, Marelli *et al.* 2011). This information is vital for planning therapeutic trials which in FRDA have been bedevilled by negative results even when large, prolonged, multi-centre designs have been employed. It is therefore important to know which scales are appropriate to the patient group, which are sensitive to change, how different subgroups of patients behave within those scales and what the magnitude of random variation and systematic progressive deterioration is according to these measures.

Section 2.3.11 above shows how four of the rating scales varied over the 2-year period of the study (SARA, INAS, ADL and SDFS). Of these, the SARA was most exhaustively analyzed as the cross-sectional results were most promising for this measure. The UK wing of the EFACTS project which encompassed 167 genetically proven cases of FRDA, 125 of whom were re-assessed after 1 year (FU1) and 116 after 2 years (FU2), was able to show statistically significant progression within the SARA both at the FU1 and FU2 visits. However, the difference between the two means over that period was only 1.33 points on the SARA scale, representing an increase from 23.2±10.2 to 24.6±9.5 and an annual rate of change of 0.7 SARA points (out of a possible maximum value of 40). Within this deterioration, there was enormous variation. Indeed, over the 2-year study

period 26.7% of the patients improved in terms of their total SARA score, 12.1% did not change and only 61.2% deteriorated.

Within the SARA, progression was most marked within those subscores which assess lower limb function, despite the fact that these subscores are heavily skewed toward their higher values. It is therefore reasonable in clinical trials to recruit patients with less lower limb involvement such as those who remain mobile. It is less advisable to use measures which exclude or place less emphasis on lower limb involvement.

Although there is some suggestion from the cross-sectional data that clinical progression plateaus when measured by the SARA (see Figure 46(B)), the longitudinal data show that progression is independent of disease severity. Figure 47 combines the cross-sectional and longitudinal data for the SARA giving an idea of overall disease progression and the considerable variation within this from individual patients.

The INAS count was insufficiently sensitive in this study to detect change over the two year period of the study. Indeed, only 37% of patients showed deterioration in this measure. Figure 17 shows that six of the sixteen count parameters are barely populated for FRDA patients which may partly explain its inappropriateness as a measure of disease severity in FRDA. The method of calculation of the count also minimizes sensitivity to change. Although many of the parameters are assessed on a 4-point semi-quantitative scale, these results are combined into simple binary scores of presence or absence for calculation of the final score. Although this has the undoubted benefit of simplifying the scale for statistical manipulation and presentation of results, it has the effect of reducing its sensitivity to change. This study therefore does not support the choice of the INAS count as an outcome measure to assess clinical change in FRDA.

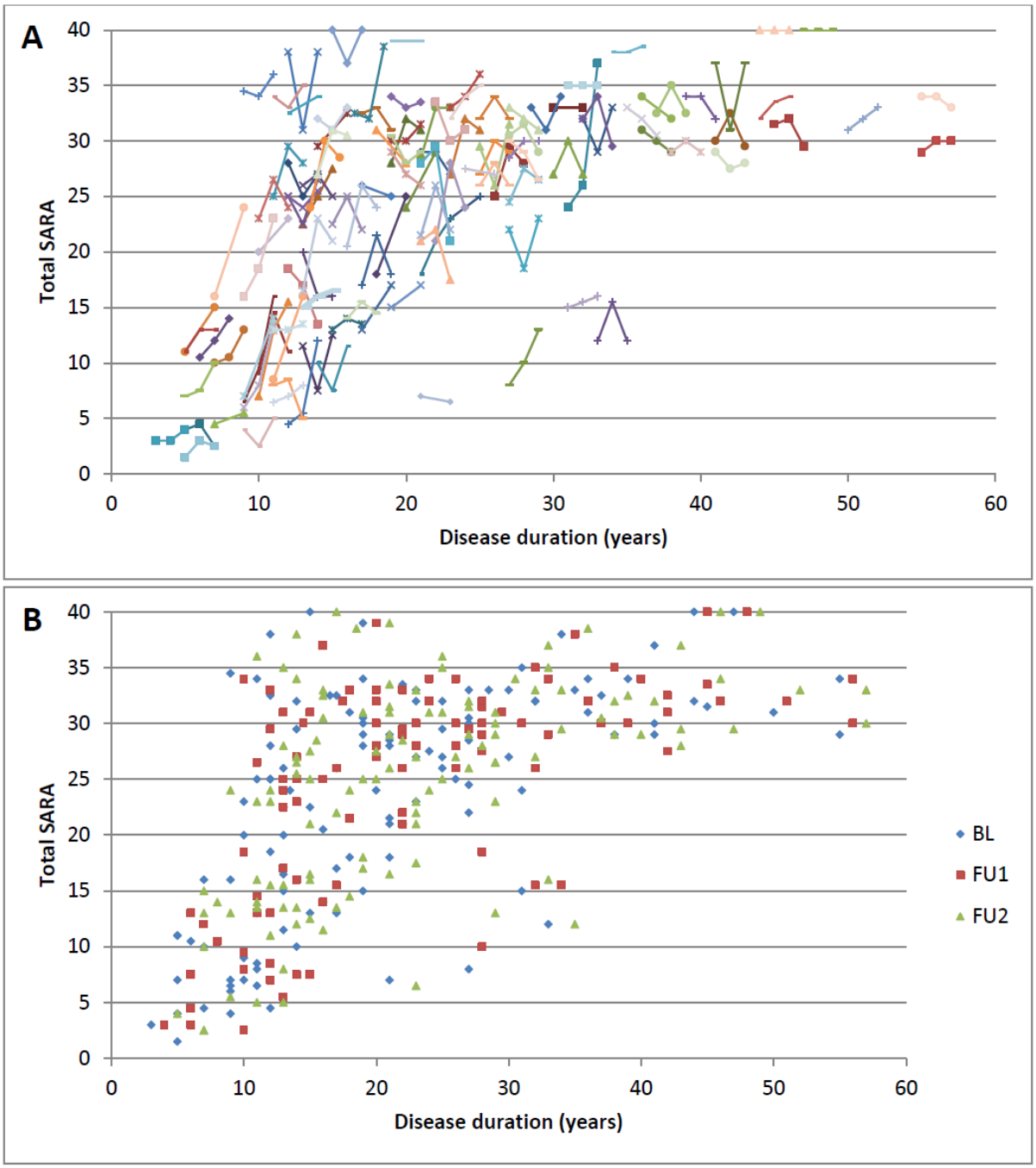


Figure 47: Total SARA values at different disease durations shown (A) by individual patient, and (B) by visit

The total ADL was able to detect progression over both one and two years of the study with a mean difference of 1.07 between BL and FU1, and 2.04 between BL and FU2 (out of a possible maximum value of 36). As with the total SARA and unlike the INAS count, a far greater proportion of patients deteriorated (66.4%) than improved (21.1%). No evidence of ceiling effect was seen and in the cross-sectional data, there was a good spread of values within most of the subscores (see Figure 15).

Progression was also detected by the SDFS with patients moving from lower to higher disability stages throughout the study (see Figure 35(C)&(D)). This is most marked when considering stages 1-3 together (between no functional disability and limited walking but without aid), compared to stages 5-6 together (between walking with two sticks and confined to bed). Over the two years of the study, the proportion of patients in stages 1-3 drops from 20.4% at BL, to 13.8% at FU1 and 10.1% at FU2. By contrast, the proportion of patients in stages 5-7 increases from 70.7% at BL, to 78.3% at FU1 and 81.4% at FU2. There is very little change overall in stage 4 (walking with one stick). Similarly, in the Wilcoxon signed ranks test, there are 16 positive and 2 negative rank changes between BL and FU1, and 28 positive and 1 negative rank changes between BL and FU2. The SDFS is therefore a good overall marker of disability which is sensitive to change over 1-2 years but provides very little scope for further analysis of the reasons for change.

It is interesting to speculate as to why a significant proportion of patients with what is regarded as a relentlessly progressive condition, should in fact fail to progress within the clinical measures within this study. The majority of patients in the study were also seen as NHS patients within the Ataxia Centre of the NHNN and so are engaged with a tertiary centre of excellence in the management of ataxia, have their drug treatments optimized and are frequently referred for specialist physiotherapy, occupational therapy, speech and language therapy and other interventions. Many of the remaining patients were also seen at specialist centres around the country. Several patients were engaged in therapeutic drug trials during the study, including those of idebenone, nicotinamide and pioglitazone. Many patients were taking co-enzyme Q_{10} or other dietary supplements and several started these agents during the period of the study. The study was purely observational with the express objective of establishing a registry from which patients could be recruited into therapeutic trials, and so participation in such trials and use of medicinal agents was entirely consistent with the ethos of the study. An alternative approach is to exclude these patients from calculations or subject the data to stratification or multivariate analysis, all of which risk diminishing the power of the study.

Patients were quite often seen by chance in the aftermath of a recent fall, fracture, infection, period of unwellness or hospital stay, giving a confoundingly suppressed baseline assessment after which they would recover either with or without active rehabilitation, and appear to have improved clinically at subsequent assessments. Such temporary effects are difficult to capture and quantify in natural history studies. The intention of recruiting such a large sample with assessments over such a long period is to attenuate these natural variations, but the present study would seem to indicate that these effects are considerable when compared to the exceptionally slow underlying progression of the disorder. Of course, the data presented in this thesis represent a fraction of a wider European study which has recruited more than 600 patients and will assess patients over at least three years and informally beyond, which should help to minimize these effects.

The majority of disease-modifying treatments for neurodegenerative disorders are aimed at minimizing or stabilizing cellular and hence clinical deterioration. Therefore, with the exception of actively regenerative interventions such as gene or stem cell therapy, future therapeutic trials will continue to be tested for their ability to show a reduction in an already exceptionally slow decline against considerable background variation. This may be more than any of the rating scales investigated in this project is capable of. One possible solution to this problem is to develop one or more biomarkers which reliably track disease activity in the long run but which react more rapidly in the short term. The wider EFACTS project may provide useful evidence in this regard as the blood and urine samples derived from this study will be subjected to proteomic analysis. Frataxin protein and mRNA levels will also be quantified. These are perhaps the most biologically plausible markers of disease activity in FRDA but have hitherto not formed part of the routine evaluation of FRDA patients in therapeutic trials. Early studies of the anti-oxidant idebenone used markers of oxidative stress such as plasma dihydroxybenzoic acid levels after salicylate administration and urinary 8-hydroxy-2'-deoxyguanosine levels (Schulz *et al.* 2000), or markers of oxidative phosphorylation such as phosphocreatine recovery after exercise measured by 31-phosphorus magnetic resonance spectroscopy (Schöls *et al.* 2001). A recent study of the histone deacetylase inhibitor, nicotinamide, using patients included in this study,

showed significant increases in protein and mRNA levels of frataxin, although no clinical changes were detected in this exploratory study because of its necessarily short duration and small size (Libri et al. 2014). However, this holds hope for future studies which is essential if large amounts of money and resources are not to be expended, and patients not put to the inconvenience and expense of attending studies which run the risk of falsely rejecting effective treatments because of the non-responsiveness of clinical rating scales.

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Chapter 3 : Friedreich's Ataxia Point Mutations & Deletions

3.1 Introduction

This chapter describes the clinical presentation of patients with compound heterozygous *FXN* point mutations and deletions both recruited as part of and outside the EFACTS project, with particular attention on the only deletion found as part of the UK wing of the EFACTS project. It also describes a project to determine the frequency of *FXN* deletions amongst patients referred to the Neurogenetics service at the NHNN with suspected FRDA.

3.1.1 *FXN* Compound Heterozygotes with Point Mutations

Whilst most patients with FRDA are homozygous for pathological GAA expansions in the *FXN* gene, approximately 4% show compound homozygosity for a point mutation in the *FXN* gene combined with a pathological GAA expansion (Campuzano *et al.* 1996). More than forty pathogenic point mutations have been described spanning all five exons of the principal gene transcript (collated by the Human Gene Mutation Database (HGMD), University of Cardiff, http://www.hgmd.cf.ac.uk/ac/index.php). These have included missense mutations caused by base pair substitutions and an in-frame insertion-deletion. There are also null mutations caused by base pair substitutions in the start codon resulting in incorrect translational initiation, as well as nonsense insertions, deletions and indels causing frame-shift variants resulting in premature termination. Intronic variants have also been described at all four splice-sites. Their location relative to the *FXN* gene exons and frataxin protein secondary structural elements is illustrated in Figure 49 (Forrest *et al.* 1998, Cossée *et al.* 1999, Pook *et al.* 2000, De Castro *et al.* 2000, Gellera *et al.* 2007, Galea *et al.* 2015, Heidari *et al.* 2013). No homozygous *FXN* point mutations have yet been described.

Frataxin is a nuclear-encoded protein which is translated in the cytoplasm as a 23.1kDa 210 amino acid species sometimes called human frataxin precursor. The amino-

terminal residues are likely to act as a mitochondrial-targeting sequence and are cleaved proteolytically in the mitochondria by the mitochondrial processing peptidase (MPP). Several cleavage sites have been demonstrated under different experimental conditions including those after residues 55 and 77. However, it is likely that processing occurs *in vivo* in humans via a two-step process. This involves cleavage of the mitochondrial signal peptide (residues 1-42) to produce a 18.8kDa 168 amino acid intermediate protein. Subsequent cleavage of the intermediate N-terminal tail (residues 42-81) produces the functional, mature 14.2kDa 130 amino acid protein (Branda *et al.* 1999, Cavadini *et al.* 2000, Condò *et al.* 2007, Schmucker *et al.* 2008). The mature protein localizes to the inner mitochondrial membrane. Expression is ubiquitous but highest in the heart and spinal cord, intermediate in the cerebellum, liver, skeletal muscle and pancreas, and low in other organs including the cerebral cortex (Campuzano *et al.* 1996). Reduced frataxin expression affects mitochondria-rich cells such as cardiomyocytes, sensory neurones of the dorsal root ganglia (DRG), pancreatic islet β-cells and cells of the cerebellar dentate nuclei and spinocerebellar tracts.

Frataxin plays a role in iron-sulphur cluster (ISC) assembly, haem biosynthesis and intracellular iron homoeostasis. These functions are undertaken by interaction with cellular proteins. Frataxin interacts with the cysteine desulphurase enzyme IscS which catalyzes the conversion of cysteine to alanine producing a highly reactive sulphide species which interacts with ionic iron to form the ISC. Frataxin also interacts with the scaffold protein IscU on which the ISC assembles (Prischi *et al.* 2010, Pandolfo & Pastore 2009). Furthermore, an extramitochondrial form of mature frataxin has been found to interact with the bifunctional ISC-dependent cytosolic aconitase/iron regulatory protein-1 (IRP1) (Condò *et al.* 2010). There is evidence that frataxin has a role in reducing oxidative stress. One of the principal fucntions of ISCs is to participate in reversible redox reactions because of the variable valency of ironic iron (Fe^{2+}/Fe^{3+}). Complexes I, II and II of the mitochondrial electron transport chain, as well as the tricarboxylic acid cycle enzyme aconitase, all contain iron-sulphur clusters which exploit this property (Lill 2009, Johnson *et al.* 2005). Frataxin also interacts with ferrocheletase, a mitochondrial protein able to mediate insertion of iron into the

porphyrin precursor as part of haem biosynthesis (Lesuisse *et al.* 2003). The significance of this in FRDA is not fully understood as failure of haem synthesis is not recognized as a major feature of the condition.

The mature frataxin protein consists of a large, twisted β-sheet composed of at least six anti-parallel β-strands ($\beta_{1\text{-}6}$), flanked by two α-helices (α_1 & α_2). The β-sheet occurs between residues 122 & 181. The amino-terminal α_1 helix occurs between residues 95 & 113, and the second α_2-helix between residues 182 & 194, with a carboxy-terminal tail formed by residues 194 onwards. The two α-helices lie roughly parallel to each other with the carboxy-terminal tail filling the groove between them (see Figure 48). One surface of the β-sheet is therefore exposed and the other in close apposition with the α-helices and carboxy-terminal tail (Musco *et al.* 2000, Dhe-Paganon *et al.* 2000). The α-helices contain a large proportion of anionic residues and may be involved in binding to iron. By contrast, the β-sheet is largely composed of uncharged residues and may be involved in protein-protein interactions (Santos *et al.* 2010, Adinolfi *et al.* 2002).

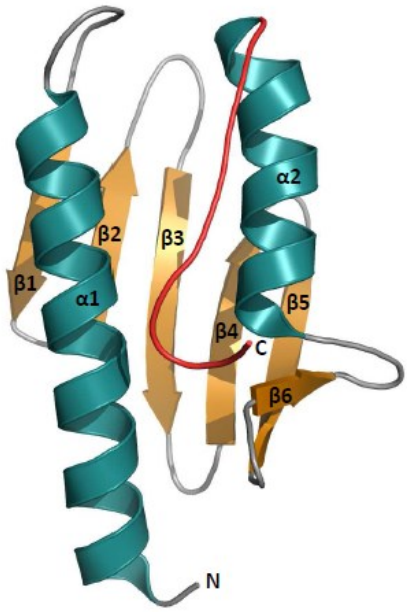


Figure 48: Ribbon representation of human frataxin
α-helices shown in turquoise, β-strands in gold and C-terminal tail in red.
Figure from Galea *et al.* 2015

Figure 49 shows all the point mutations hitherto described in the *FXN* gene. It can be seen that all the missense mutations result in changes within the mature frataxin

protein and none causes alterations of the cleaved amino-terminal elements. The vast majority therefore occur in the terminal three exons. By contrast, most nonsense mutations occur within the first three exons including many in those portions of the gene encoding the cleaved amino-terminal elements. This is presumably because changes in the amino acid sequence of those portions of the protein which will ultimately be cleaved, do not affect the function of the final protein and so may be relatively tolerated. On the other hand, mutations which cause truncation of the protein both within the cleaved mitochondrial-targeting sequence and the final mitochondrially expressed molecule, will be deleterious; truncation of the terminal portion of the mitochondrial protein seems not be so harmful.

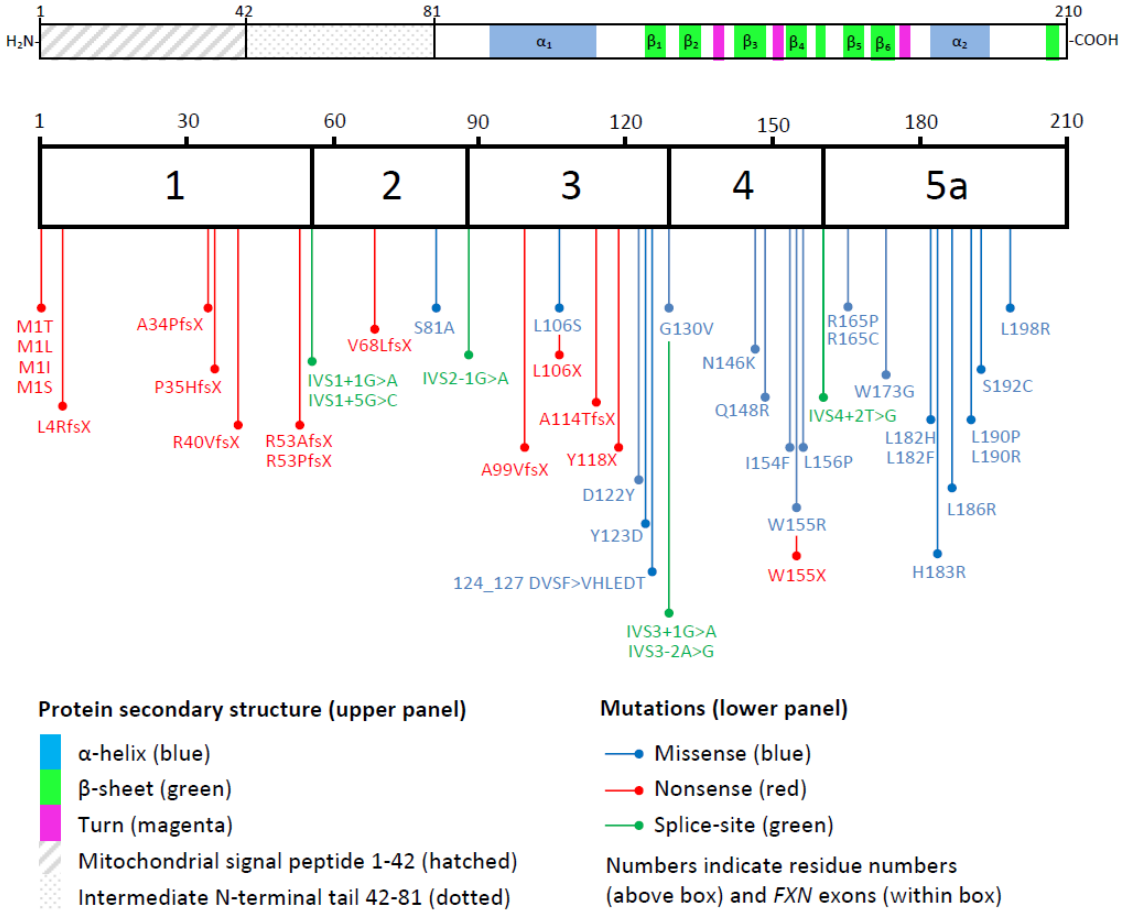


Figure 49: Location of point mutations relative to exons of the *FXN* gene and secondary structure of the frataxin protein

Genetic data from Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), Gellera *et al.* (2007), Galea *et al.* (2015) and original papers. RefSeq NM_000144.4, NP_000135.

Protein structural data from UniProtKB (www.uniprot.org). ID: Q16595. PDB: 3T3L

Campuzano and colleagues described the first three *FXN* point mutations together with the initial identification of the *FXN* GAA triplet repeat expansion (Campuzano *et al.* 1996). These included missense (p.I154F), nonsense (p.L106X) and splice-site (g.IVS3-2A>G) mutations. Bidichandani *et al.* (1997) described the fourth, and to this date, commonest mutation (p.G130V) which they found resulted in an atypically mild clinical phenotype with very slow progression, no dysarthria and little or no incoordination. Cossée and co-workers then described the first of a number of mutations in the start codon (c.3G>T, p.M1I) that cause improper translational initiation (Cossée *et al.* 1997a). At that point, Forrest and colleagues compared a small series of five *FXN* compound heterozygotes including three further novel mutations (p.R165C, p.L182F & g.IVS4+2T>G) with those already described in the literature (Forrest *et al.* 1998). They concluded that splice-site, nonsense and initiation codon mutations which cause absence of functional frataxin were associated with a severe phenotype, but that missense mutations, even in highly evolutionarily conserved residues, cause a mix of mild and severe phenotypes.

Cossée *et al.* (1999) compared the clinical features of 25 compound heterozygotes from 19 families, with 196 patients homozygous for the GAA repeat expansion. Patients with truncating mutations, or missense mutations in sequences encoding the carboxy-terminal half of the frataxin protein, had a similar phenotype to those with a homozygous GAA expansion. However, patients with a missense mutation in sequences encoding the amino-terminal half of the frataxin protein (D122Y and G130V) had a milder clinical phenotype with slow disease progression, early-onset spastic gait, retained upper and lower limb reflexes, no dysarthria and little or no ataxia. Compound heterozygotes also showed increased prevalence of optic disc pallor, an observation which has not subsequently been replicated.

Galea and collaborators (2015) have published the largest series to date of *FXN* compound heterozygotes including cases from the present thesis (Galea *et al.* 2015). They studied the effect of *FXN* point mutation on (i) the tertiary structure of the frataxin protein by molecular modelling based on the known crystal structure of the protein; (ii) frataxin stability using a series of *in silico* structure-based protein stability prediction programs; and (iii) protein function, iron binding and protein-protein

interactions referring to previously published experimental data. They found that residues buried within the interior of the protein form a tightly packed hydrophobic core which helps to stabilize the protein structure. Mutations affecting residues within the hydrophobic core had the greatest impact on protein stability by generating steric clashes in the case of substitution by larger residues; cavities in the case of substitution by smaller residues; and unfavourable interactions in the case of substitutions of hydrophobic residues for charged ones. Null mutations caused the most severe phenotype but the clinical effect of particular missense mutations depended on factors acting at each individual site including effects on the tertiary structure of the protein, its ability to interact with other species and hence the overall functionality of the molecule.

The majority of *FXN* point mutations described have been private mutations; cases have been reported from Asia, Australia, the South Pacific, USA and throughout Europe (Gellera *et al.* 2007). However, certain mutations have been reported recurrently. These include the following missense mutations: p.G130V (Bidichandani *et al.* 1997, Forrest *et al.* 1998, Cossée *et al.* 1999), p.I154F (Campuzano *et al.* 1996, Filla *et al.* 1996, Cossée *et al.* 1999, Gellera *et al*. 2007), p.R165C (Forrest *et al.* 1998, McCormack *et al.* 2000) and p.W173G (Cossée *et al.* 1999, Gellera *et al*. 2007). It may be that some of these cases share a common founder, and that, for example in the case of p.G130V described above, a milder clinical phenotype with later onset allows the generation of progeny and the consequent propagation of this mutation in the population. Certain mutational hot-spots have also been identified. For example, five different nucleotide changes in the start codon (c.1A>C, c.2T>C, c.2delT, c.3G>T & c.3G>A) result in four different amino acid changes in the first residue (p.M1L, p.M1T, p.M1S & p.M1I) all causing incorrect translational initiation (Cossée et al. 1999, Zühlke *et al.* 1998, Potter *et al.* 2000, Zhu *et al.* 2002). At nucleotide 317, three different sequence changes (c.317T>G, c.317delT & c.317T>C) cause either nonsense mutations because of the direct generation of a stop codon (p.L106X) or a pathogenic missense mutation (p.L106S) (Campuzano *et al.* 1996, Bartolo *et al.* 1998, Pook *et al.* 2000).

DeCastro *et al.* (2000) looked for pathological GAA expansions in the *FXN* gene amongst 241 patients with early-onset spinocerebellar ataxia. They found 175 patients

with a GAA expansion including seven (4%) with just one expansion. Of these, four were compound heterozygous for a point mutation including three nonsense mutations (p.W155X, p.E100fsX & p.R39fsX) and a splice-site variant (g.IVS3-2A>G). All were of Spanish or Cuban origin. The authors noted that three of the patients (those with p.W155X, p.R39fsX & g.IVS3-2A>G) clinically had classical FRDA with early onset between the ages of 3 & 4, whereas the last (p.E100fsX) had late-onset disease and was still ambulant after 12 years. The first three all had GAA expansion sizes of between 800 and 900 repeats, whereas the milder atypical case had a much smaller expansion of 250 repeats. They concluded that the size of the single pathological GAA expansion in a compound heterozygote continues to determine disease severity particularly in those who share nonsense mutations.

Gellera and colleagues also compared age at onset with GAA expansion size amongst 13 compound heterozygotes bearing *FXN* point mutations and found a statistically significant inverse correlation which was even stronger when only the nonsense mutations were considered. Furthermore, there was also a statistically significant inverse correlation between GAA expansion size and frataxin expression as measured by Western blot analysis of lymphoblastoid cell lines derived from these patients, although only after two outliers were excluded from analysis (Gellera *et al.* 2007). In the same restricted sample, age at onset positively correlated with frataxin levels. Taken together, these results suggest that even in the presence of a point mutation, GAA expansion size remains one of the principal determinants of frataxin production which in turn determines clinical severity. This is particularly marked in the case of nonsense mutations in which the allele bearing the GAA expansion is presumably solely responsible for frataxin production.

Thus, a large range of point mutations in the *FXN* gene has been described. Null mutations which are usually clustered in the first three exons of the gene, have the most severe clinical effects. Missense mutations usually occur in the terminal three exons and are rarely found in those parts of the gene which encode the peptide sequences which are cleaved before mitochondrial expression. Missense mutations have variable effects depending upon their effect on the tertiary structure of the protein, its ability to interact with other molecules and overall function. The size of the

GAA expansion on the other allele remains an important determinant on clinical severity, particularly in the presence of null mutations.

3.1.2 *FXN* Compound Heterozygotes with Large Deletions

The development of methods for measuring gene dosage such as multiplex ligation-dependent probe amplification (MLPA) has enabled the identification of compound heterozygotes with large *FXN* deletions (Deutsch *et al.* 2010). Eleven such cases have thus far been described some of which have atypical features. Zühlke and colleagues described a 2776 base pair deletion including exon 5a of the *FXN* gene using a quantitative duplex PCR assay with exon-specific oligonucleotide probes analogous to MLPA, followed by sequencing of the 5a exon (g.120032_122808del). The individual concerned had typical features of FRDA and progression to wheelchair use over 6 years (Zühlke *et al.* 2004). Deutsch and co-workers described an individual with a deletion in exons 2-3 which had typical features except rapid progression to wheelchair use in 4 years and no dysarthria (Deutsch *et al.* 2010). The same group mention *en passant* a second unpublished case of an 11-year old boy with a compound heterozygous deletion of exon 5 and classical disease phenotype although a severe presentation. His younger brother also developed gait disturbance and loss of deep tendon reflexes (Brigatti *et al.* 2012). Van den Ouweland and others described a large *de novo* deletion of between 1,603kbp and 2,477kbp including complete deletion of the *FXN* gene and possibly contiguous genes. The exact extent of the deletion could not be determined because of poor probe coverage. The patient had an abnormal presentation with chorea and intention tremor but slow progression over nearly 30 years (van den Ouweland *et al.* 2012).

The largest series to date was published by Anheim and colleagues in a large collaboration of specialist centres in France (Anheim *et al.* 2012). They described six cases from five families clustered in two sites in Brittany and the South of France. Four cases involved deletions of exons 4 & 5, one involved only exon 4, and one exons 2 & 3. The familial case involved a great-aunt and great-niece who shared the same exon 4/5 deletion but had inherited GAA expansions from unrelated relatives resulting a form of pseudo-dominant inheritance. Anheim's cases typically had young onset

(range 3-12) with severe neurological phenotype, usually presenting with unsteadiness. Half also had diabetes, 80% scoliosis and 83% cardiomyopathy including one case of severe angina at 9 years old.

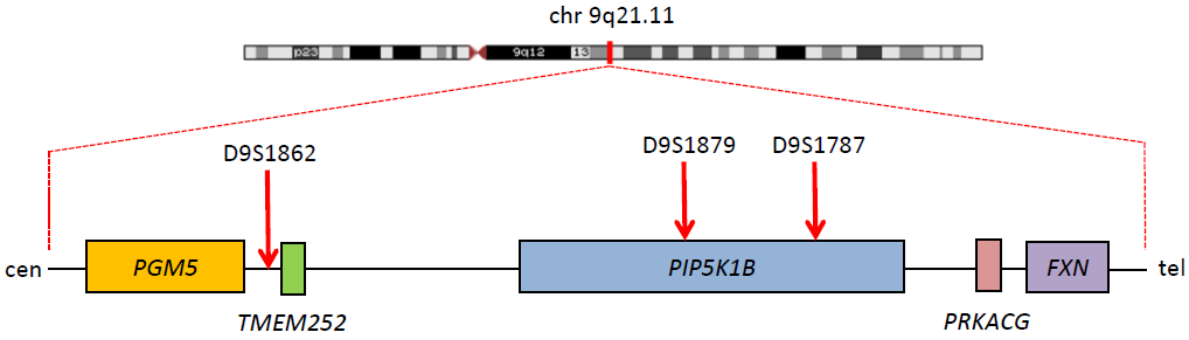
The only systematic study of exonic *FXN* deletions has occurred in the Norwegian population (Wedding *et al.* 2015). This study contacted all 22 public hospitals in Norway which have a paediatric or neurology unit, the three laboratories that undertake *FXN* gene testing, patient support organizations and the national centre for rare diagnoses. The hospitals' electronic coding records were searched by diagnostic category and the project was presented at the national neurological meeting. Twenty-seven patients had genetically confirmed FRDA with homozygous GAA expansions. A further four had clinical ataxia and one GAA expansion. Of these, one was heterozygous for a *FXN* point mutation and a further one had a large deletion. Of the remaining two, one had an *OPA1* mutation and the last currently has no genetic diagnosis. The *FXN* deletion spanned 2776 base pairs around exon 5a and was identical to that described by Zühlke *et al.* (2004). Clinically, the patient had typical presentation with onset at age 7 with unsteadiness and progression to use of a wheelchair. There was cardiac involvement and scoliosis but no diabetes.

No homozygous exonic *FXN* deletions have yet been reported. Furthermore, no examples of *FXN* multiplication are known.

3.1.2.1 Clinical Case: Patient D

During the assessment of the patients for the EFACTS project, it became apparent that there was a discrepancy between the genetic diagnosis of one of the study patients recorded in EFACTS, and that reported by the patient himself. Genetic testing for the study patients was undertaken by the Université Libre de Bruxelles as described in Section 2.2.4 of Chapter 2 above. Patient D was reported as having two homozygous GAA expansions of 785 repeats (and hence no normal-sized GAA sequence). The patient reported that he was a compound heterozygote for a GAA expansion and a deletion and had paperwork supporting this. He had previously been assessed by the Clinical Genetics Service of the Royal Devon and Exeter Hospital who were able to supply further information.

They had also initially thought that he had homozygous pathological GAA expansions but testing of his mother, who also had neurological symptoms, revealed no evidence of an expanded allele by long-range PCR, triplet-primed PCR or Southern blotting. He had therefore not inherited a pathological GAA expansion from her. It was unlikely that he had a *FXN* gene point mutation as these are always accompanied by a normal-sized GAA sequence on the same allele and an expanded sequence on the other allele, making the patient a compound heterozygote. They hypothesized that she might carry a pathological deletion encompassing the GAA repeat sequence. They analyzed three microsatellite markers neighbouring the *FXN* gene, *viz* D9S1787, D9S1879 and D9S1862. D9S1787 (also known as AFMA142YD1) is 154.5kbp centromeric to the start of the *FXN* gene. D9S1879 (also known as AFMA070WH1) is 187.7kbp centromeric to the start of the *FXN* gene. Both of these markers lie within the phosphatidylinositol-4-phosphate-5-kinase type 1β (*PIP5K1B*) gene. D9S1862 (also known as AFMC013YH5) lies 505.9kbp centromeric to the start of the *FXN* gene between the transmembrane protein 252 (*TMEM252*) and phosphoglucomutase-5 (*PGM5*) genes. Figure 50 shows the location of the markers and their relation to the *FXN* gene. Both mother and son were heterozygous for these markers excluding a deletion encompassing genes upstream of but some distance from the *FXN* gene.



PGM5: Phosphoglucomutase-5
TMEM252: Transmembrane protein 252
PIP5K1B: Phosphatidylinositol-4-phosphate-5-kinase type 1β
PRKACG: Protein kinase cAMP-dependent catalytic γ

Figure 50: Location of microsatellite markers relative to *FXN* gene

They then developed a quantitative fluorescence PCR assay following a method previously used to detect exonic deletions in cases of Becker and Duchenne muscular dystrophy (Yau et al. 1996). They used the primers GAA-104F (5'-GGC TTA AAC TTC CCA CAC GTG TT-3') and GAA-629R (5'-AGG ACC ATC ATG GCC ACA CTT-3') which flank the

GAA sequence and have previously been used to determine GAA repeat length (Filla et al. 1996) but are different from those used in the laboratories at NHNN and the Université Libre de Bruxelles (Campuzano et al. 1996). The exact binding sites of these primers are shown in Figure 59. Primers were also used against a reference sequence in exon 9 of the glucokinase gene *GCK* on chromosome 7p15.3-15.1, mutations in which cause maturity-onset diabetes of the young type 2 (MODY2). Control samples were used from individuals with homozygous normal-sized GAA sequences. Dosage quotients (DQ) were calculated by comparing the *FXN/GCK* peak area ratios of the patient and his mother with those of controls. These were consistent with heterozygous deletions in both. Exact characterization of the breakpoints was not undertaken (Thomson *et al.* 2003)(Martina Owens, personal communication).

This case is interesting as it involves a *FXN* gene deletion. Very few cases have been reported and the clinical phenotype is not fully characterized. Although they are assumed to be rare, no systematic studies of their prevalence have been undertaken. The finding on long-range PCR of a single band corresponding to a pathological GAA expansion and no normal-sized band, is interpreted by most diagnostic laboratories as implying that the patient has two equal-sized GAA expansions. This case shows that the absence of a normal-sized band does not necessarily imply that the patient has two pathological expansions which potentially is important in the context of genetic counselling and has implications in understanding underlying pathological mechanisms in FRDA. Fifteen of the 147 patients in the UK wing of EFACTS (10.2%) for whom GAA sizing was available, were reported by the laboratory of the Université Libre de Bruxelles as having equally sized pathological GAA expansions. The above observation is therefore relevant to all these patients.

3.2 *FXN* Compound Heterozygotes with Point Mutations

3.2.1 Method

The database of the Neurogenetics Department of the NHNN and the EFACTS Registry were searched for known cases of compound heterozygotes with point mutations (NHNN search undertaken by Dr Mary Sweeney & Dr Robyn Labrum). Clinical data

were obtained from the patients' notes and the EFACTS Registry as described in Chapter 2. Testing for repeat expansions was undertaken in the Neurogenetics laboratory of the NHNN as described in 3.3.1 below, and in the laboratory of the Université Libre de Bruxelles as described in Chapter 2. Sequencing for point mutations was undertaken in the Merseyside and Cheshire Regional Molecular Genetics Laboratory, Liverpool Women's Hospital, Liverpool, UK and the laboratory of the Université Libre de Bruxelles. Both laboratories use the same previously published technique (Pandolfo 2006) using the following primers:

Exon 1:	5'-AGC ACC CAG CGC TGG AGG-3' (forward)
	5'-CCG CGG CTG TTC CCG G-3' (reverse)
Exon 2:	5'-AGT AAC GTA CTT CTT AAC TTT GGC-3' (forward)
	5'-AGA GGA AGA TAC CTA TCA CGT G-3' (reverse)
Exon 3:	5'-AAA ATG GAA GCA TTT GGT AAT CA-3' (forward)
	5'-AGT GAA CTA AAA TTC TTA GAG GG-3' (reverse)
Exon 4:	5'-AAG CAA TGA TGA CAA AGT GCT AAC-3' (forward)
	5'-TGG TCC ACA ATG TCA CAT TTC GG-3' (reverse)
Exon 5a:	5'-CTG AAG GGC TGT GCT GTG GA-3' (forward)
	5'-TGT CCT TAC AAA CGG GGC T-3' (reverse)

This technique screens for mutations in exons 1-5a and so covers the entire coding region of the most commonly expressed *FXN* transcript. It does not exclude pathogenic mutations in the promoter region, the alternative exon 5b, the untranslated exon 6 or the 3'-untranslated region, and does not exclude cryptic splice site mutations or somatic mosaicism.

3.2.2 Results

Thirteen examples of compound heterozygotes with point mutations were found, including three pairs of siblings. Of the thirteen, seven were included in EFACTS including two of the pairs of siblings. The genetic and basic demographic data relating to all these patients are detailed in Table 27. The location of the mutations is shown in Figure 51. Unfortunately, very little clinical information was available for patients 11 to 13 which are therefore not included in the ensuing discussion or analysis.

Table 27: Basic demographic & genetic details of *FXN* compound heterozygotes

Patient no.	Mutation	Protein change	Exon	Mutation type	Effect	Protein truncating	GAA1 size	GAA2 size	Gender	Family history	Age at exam	Age at onset	Disease duration	Age at wheelchair-bound	In EFACTS
1	c.389G>T	p.Gly130Val	4	S	MS	NT	67	1100	F	Sibling of 2	48	13	35	N/A	Yes
2	c.389G>T	p.Gly130Val	4	S	MS	NT	NK	NK	M	Sibling of 1	50	10	40	N/A	Yes
3	c.389G>T	p.Gly130Val	4	S	MS	NT	30	612	M	No	41	15	26	N/A	Yes
4	c.389G>T	p.Gly130Val	4	S	MS	NT	45	745	M	Sibling of 5	50	13	37	30	Yes
5	c.389G>T	p.Gly130Val	4	S	MS	NT	NK	NK	F	Sibling of 4	46	15	31	30	No
6	c.389G>T	p.Gly130Val	4	S	MS	NT	10	867	M	No	27	15	12	N/A	Yes
7	c.389G>T	p.Gly130Val	4	S	MS	NT	NK	NK	F	Sibling of 11	28	13	15	N/A	No
8	c.389G>T	p.Gly130Val	4	S	MS	NT	NK	NK	F	Sibling of 10	22	19	3	N/A	No
9	c.493_494 CG>GA	p.Arg165Asp	5a	S	MS	NT	6	867	M	Yes (cousin)	23	12	11	N/A	Yes
10	c.2T>C	p.Met1Thr	1	S	II	T	NK	NK	F	No	21	16	5	N/A	Yes
11[a]	c.389G>T	p.Gly130Val	4	S	MS	NT	NK	NK	F	NK	NK	NK	NK	NK	No
12[b]	c.2T>C	p.Met1Thr	1	S	II	T	NK	NK	F	NK	NK	NK	NK	NK	No
13[c]	c.357_378 dup22	p.Phe127fsX	3	D	NS	T	NK	NK	M	NK	NK	NK	NK	NK	No

Very minimal clinical information available for cases 11 to 13 (see below). Consequently not included in subsequent tables

[a]Ataxia

[b]Progressive ataxia & cardiomyopathy

[c]Progressive ataxia, absent ankle reflexes, MRI brain & spine normal

S=base substitution; D=deletion; MS=missense; NS=nonsense; II=incorrect initiation; T=truncating; NT=non-truncating; NK=not known; N/A=not applicable

RefSeq: NM_000144.4, NP_000135

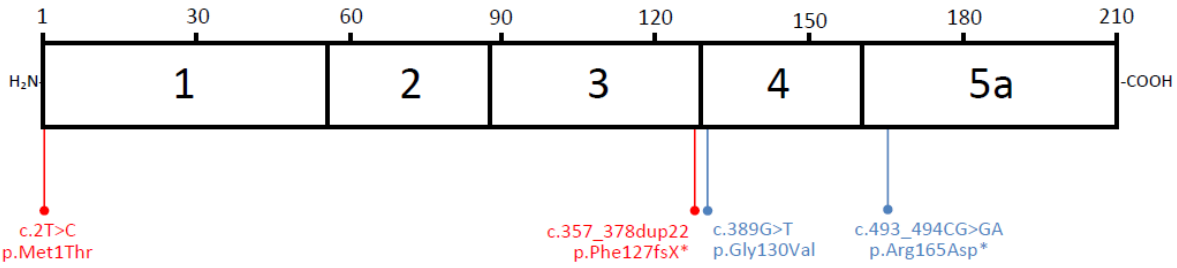


Figure 51: Location of FXN mutations described in this study

Nonsense mutations (red), missense mutations (blue), novel mutations (*)
Residue numbers (above box), exon numbers (within box). RefSeq NM_000144.4, NP_000135

3.2.2.1 Genetic Studies

Nine examples were found of the previously documented c.389G>T mutation which causes a pathogenic missense mutation with substitution of valine for glycine at residue 130 in exon 4 (Bidichandani et al. 1997, Forrest et al. 1998, Cossée et al. 1999). There were two examples of the previously reported c.2T>C mutation involving a change in the start codon in exon 1 from ATG to ACG causing an amino acid change from methionine to threonine and consequently incorrect translational initiation (Cossée *et al.* 1999).

Two novel mutations were found. The first involved a duplication of a 22bp sequence (TTTGGGGTACCTCT**TGA**CTTCT) starting at nucleotide 357 at the end of exon 3. This causes a frameshift mutation starting at residue 127 and resulting in a premature stop codon after 12 codons because of the previously out-of-frame TGA codon within the duplicated sequence (in bold above). Such a nonsense mutation is considered obligately pathogenic. No duplication mutations have previously been reported in FRDA, and no mutations have previously been reported at this location. Unfortunately, the only clinical information available for the patient was that she had progressive ataxia with absent ankle reflexes and normal MR imaging of the brain and spine. She was a five year old child when tested and had symptoms which had progressed over at least one year.

The second novel mutation involved a dinucleotide substitution (or short indel) at positions 493 & 494. This causes a missense mutation with substitution of aspartic acid for arginine. These two positions form a mutational hot-spot with two different single-nucleotide mutations already described, causing different amino acid substitutions at the same residue, *viz.* c.493C>T/p.Arg165Cys (Forrest et al. 1998, McCormack et al. 2000) and c.494G>C/p.Arg165Pro (De Michele *et al.* 2000, Ygland *et al.* 2014). The present case forms a third. The previous description of different nucleotide variants causing an amino acid substitution at this residue in the context of a clinical diagnosis of FRDA supports the pathogenicity of the present mutation although is not specific for this amino acid change.

In silico tests of pathogenicity also provide supportive evidence. No variant was found for either position 493 or 494 in the database of the 1000 Genomes Project, the Exome Sequencing Project (ESP) or the Database of Single Nucleotide Polymorphisms (dbSNP), indicating the rarity of variants at these positions in the general population and supporting the pathogenicity of the present mutation. The Genome Evolutionary Rate Profiling (GERP) score was 5.07, the Phylogenetic Analysis with Space/Time models Conservation Program (PhastCons) score was 1 and the Phylogenetic p-values (PhyloP) score was 5.515. These values each indicate conservation over evolutionary time at this position which supports the pathogenicity of the variant. The Grantham score is 65 which is only a moderately conservative change. The Sorting Intolerant From Tolerant (SIFT) algorithm predicted that the change would be damaging with a score of zero. The Polymorphisms Phenotyping version 2 (PolyPhen2) program predicted that the variant was probably damaging with a score of 0.995. The prediction of the Mutation Tasting program which incorporates multiple pathogenicity prediction techniques is that the mutation is disease-causing with a probability of 0.9999. The multiple sequence alignments generated by the program are shown in Table 28 demonstrating evolutionary conservation at these sites over multiple species. Thus, these measures provide good evidence that the mutation is pathogenic. For a more detailed description of these *in silico* techniques, see Chapter 5.

Table 28: Multiple sequence alignments for human *FXN* c.493_494CG>GA

Species	Match	Gene	aa	Alignment
Human wt			37364	G T G G A C C T A A G C G T T A T G A C T G G A
Human mutated	not conserved		37364	g t g g a c c t a a g **g a** t t a t g a c t g g a
P troglodytes	no homologue			
M mulatta	all identical	ENSMMUG00000000357	39574	g t g g a c c c a a g **c g** t t a t g a c t g g a
F catus	all identical	ENSFCAG00000008813	33434	g t g g g c c g a a g **c g** c t a c g a c t g g a
M musculus	all identical	ENSMUSG00000059363	20563	g c g g c c c c a a g **c g** c t a t g a c t g g a
G gallus	all identical	ENSGALG00000015108	8585	g t g g g c c c a a g **c g** c t a t g a c t g g a

3.2.2.2 Clinical Features

The clinical features of the ten compound heterozygotes for which adequate clinical information is available are given in Table 29 and Table 30. Table 31 summarizes the clinical findings and records the results of the clinical ratings scales (SDFS, total ADL, total SARA, INAS count & SCAFI). The clinical information is derived from the EFACTS

assessment in the case of patients 1, 2, 3, 4, 6, 9 & 10, and from the medical notes and clinic letters in the case of patients 5, 7 & 8. Consequently, the clinical rating scales were only undertaken in patients from the former group. In keeping with the main EFACTS cohort, only four out of the seven patients for whom SCAFI testing was undertaken were able to complete the 8-metre timed walked. All patients were able to complete the 9-hole peg test and the PATA test.

The mean±SD age at onset of the ten patients was 14.1±2.3 (range 10-19). The mean±SD age at examination was 35.6±12.4 (range 21-50) and disease duration 21.5±13.9 (range 3-40). There was no statistical difference in these parameters between the compound heterozygotes and the homozygous patients (see Table 32). There were also no statistically significant differences in the SDFS, total ADL and INAS count. The total SARA was significantly lower in the compound heterozygotes compared to the homozygous patients (13.7±5.6 vs 22.9±10.0, Mann-Whitney U-test, Z=-2.349, p=0.019) suggesting that they have less severe ataxia even for the same age and disease duration. However, this difference did not survive Bonferroni correction.

Various meaningful clinical features for which data were available in a form with which statistical comparison could be made between the compound heterozygotes and the homozygous patients are given in a binary form (presence/absence) in Table 31. The results of the statistical comparison of these two groups are given in Table 33. Patient 2 was excluded from the comparison of spasticity as he had such marked cogwheeling rigidity associated with a diagnosis of Parkinson's disease which made the independent assessment of spasticity impossible. He was included in other comparisons although the Parkinson's disease may have affected other parameters such as weakness, dysarthria and dysphagia, albeit to a less clearly definable extent. He had symptoms of gait instability from age 10 but was not diagnosed with FRDA until age 35. He was diagnosed with Parkinson's disease at age 42. He carries the p.Gly130Val mutation for which coexistent parkinsonism has not previously been described. Indeed, no other patient in the EFACTS cohort has a diagnosis of Parkinson's disease or clinical features of parkinsonism. Furthermore, his sister who has the same point mutation and similar age at onset, does not have evidence of Parkinson's disease. His diagnosis of Parkinson's disease is therefore presumably incidental.

From the clinical parameters summarized in Table 31, six showed statistically significant differences between the compound heterozygotes and the homozygous patients of which four survived Bonferroni correction. These were hyperreflexia (60.0% vs 4.4%, Fisher's exact test, $p<0.0005$), square wave jerks (10.0% vs 68.4%, $p<0.0005$), dysarthria (30.0% vs 90.9%, $p<0.0005$) and dysphagia (20.0% vs 74.0%, $p=0.001$). The presence of broken pursuits eye movements (40.0% vs 80.4%, $p=0.008$) and wheelchair-bound status (20.0% vs 58.2%, $p=0.023$) did not survive Bonferroni correction. Thus, it appears that compound heterozygotes have more brisk deep tendon reflexes, a lower frequency of square wave jerks and suffer less dysarthria and dysphagia than homozygotes. They may also have a lower frequency of broken pursuits and less frequently be confined to a wheelchair.

Table 29: Clinical features of compound heterozygous FRDA patients with point mutations (part 1)

Patient no.	Symptom at onset	Gait	Falls	Ataxia	Tone	Muscle cramps/ spasms	Motor deficit	Pin prick	Vibration	Proprioception	Deep Tendon Reflexes	Plantars	Dysarthria	Dysphagia
1	Instability & abnormal gait	Severe ataxic gait	Rare	UL: Slight LL: Moderate	UL/LL: NAD	Mild	UL: NAD LL: Mild	UL: NAD LL: NAD	UL: NAD LL: Mild	UL: NAD LL: Mild	UL/LL: Absent	Up	Mild	No
2	Gait instability	NK	None	Mild	UL/LL: Cogwheeling rigidity	Severe	UL: NAD LL: Severe	UL/LL: Mod	UL: Mod LL: Severe	NK	UL: Absent LL: Brisk	Up	No	No
3	Turned foot in; progressive stiffness	Severe spastic gait	None	None	UL: NAD LL: Severe	Severe	UL: NAD LL: Severe	UL/LL: NAD	UL/LL: NAD	UL: NAS LL: Mod	UL: Normal or reduced. KJ: Brisk; AJ: Absent	Up	No	No
4	Walked on toes, then feet turned in	Unable to walk	Yes	None	LL: Severe	Severe	UL: NAD LL: Severe	UL/LL: severe	UL: Mod LL: Severe	UL/LL: Mod	UL/LL: Absent	Down	Mild	No
5	Abnormal gait	Unable to walk	None	None	LL: Severe	NK	UL: NAD. LL: Severe	UL/LL: Mod	UL: NAD LL: Severe	UL: NAD LL: severe	UL: Brisk LL: Absent	Up	No	Mild
6	Spinal scoliosis	Severe ataxic gait	Rare	UL: NAD LL: Moderate	UL/LL: NAD	Severe	UL: NAD LL: Mild	UL: NAD LL: Severe	UL: NAD LL: Mod	UL: Severe LL: Mod	UL : Normal or reduced; TJ: Brisk. KJ: Brisk; AJ: Reduced	Up	No	No
7	Difficulty running	Spastic gait with bilateral foot drop	NK	UL: NAD	UL: Slight catch	NK	UL: NAD LL: Mild	NK	UL: NAD LL: Severe	UL: NAD LL: Mild	UL: Brisk KJ: Brisk AJ: Absent	Up	No	No
8	Clumsiness on playing sport	Spastic gait with scissoring	Rare	UL: NAD LL: Mild	UL: NAD LL: Mild	NK	UL/LL: NAD	NK	UL/LL: NAD	UL/LL: Mild	UL: NAD KJ: Brisk AJ: Absent	Up	No	No
9	Abnormal gait	Severe spastic-ataxic gait	None	Only visible on gait	UL: NAD LL: Severe	Severe	UL: NAD LL: Mild	UL/LL: NAD	UL: NAD LL: Mod	UL: NAD LL: Mild	UL : Absent; SJ: Brisk KJ: Brisk; AJ: NAD	Absent	Mild	No
10	Clumsiness & falls	Severe ataxic gait with hyper-extended knees	Frequent	Only visible on gait	UL/LL: NAD	Severe	UL: NAD LL: Mild	UL: NAD LL: Mild	UL/LL: NAD	UL: NAD LL: Mod	UL/LL: Absent	Up	No	Mild

NAD=No abnormality detected

UL=Upper limb; LL=Lower limb; BJ=Biceps jerk; TJ=Triceps jerk; SJ=Supinator jerk; KJ=Knee jerk; AJ=Ankle jerk

Table 30: Clinical features of compounds heterozygous FRDA patients with point mutations (part 2)

Patient no.	Hearing impairment	Vision	Broken pursuits	Square wave jerks	Nystagmus	Saccadic dysmetria	Scolioisis	Foot abnormalities	Diabetes	Cardiomyopathy	Echocardiogram	ECG	Bladder	Bowel	Neurophysiology	MR Imaging
1	No	Hyper-metropia only	Yes	No	Mild	Yes	Mild	Bilateral PC/EV	No	Yes[d]	IVSd 7mm. LVPWd 7mm. EF 60% (age 42)	SR. No RA. No LVF	Normal	Normal	NK	NK
2	No	Hyper-metropia only	Yes	Yes	No	No	No	Pes planus	No	No	NK	NK	Mild urgency	Normal	NK	NK
3	No	Astigmatism only	No	No	No	No	Mild	Bilateral PC/EV	No	No	IVSd 8mm. LVPWd 9mm. EF 63% (age 41)	NK	Mild urgency	Normal	NK	Medial right parietal cavernoma. No other intracranial abnormalities
4	Yes	Poor acuity since strabismus operation	No	No	No	Yes	No	Bilateral PC/EV	No	No	IVSd 10mm. LVPWd 9mm. EF 69% (age 48)	SR. No RA. No LVF.	Frequent urge & incont-inence	Normal	Absent SAPs with mild motor changes	NK
5	No	Normal	No	No	No	No	None	Talipes equinus	No	No	IVSd 9mm. LVPWd 9mm. EF 60% (age 46)	Normal	Normal	Normal	Sensory axonal neuropathy	Brain normal. Spinal cord slender in thoracic region.
6	No	Myopia only	No	No	No	No	Yes[c]	No	No	No	NK	Normal	Mild urgency	Normal	NK	NK
7	No	Poor[b]	Mild	No	No	No	Slight	Claw toes & moderate PC	No	No	NK	Normal	Normal	Normal	Mild length-dependent sensorimotor neuropathy	Brain normal. Cord thin throughout.
8	No	Normal	No	No	No	Mild	No	Slight talipes equinus	No	No	NK	NK	Normal	Normal	NK	NK
9	No	Normal	Yes	Yes	Yes	No	No	No	No	Yes	NK	SR. No RA. LVF	Normal	Normal	NK	NK
10	Yes[a]	Normal	No	No	No	No	Mild	Mild PC & talipes equinus	T1DM since 19	No	NK	NK	Normal	Normal	NK	NK

[a]Deaf since 3 years old (cause unknown)

[b]Poor acuity right eye secondary to ocular toxoplasmosis. No progressive visual problem

[c]Required corrective surgery aged 18

[d]Also rheumatic fever

PC=Pes cavus; EV=Equinovarus; ECG=Electrocardiogram; SR=Sinus rhythm; RA=Repolarization abnormalities; LVF=Left ventricular failure

Table 31: Summary of clinical features and clinical rating scales for compound heterozygous FRDA patients with point mutations

Patient no.	Paresis	Spasticity	Hyperreflexia	Areflexia	Extenor plantars	Vibrational sensory loss	Broken pursuits	Square wave jerks	Diabetes	Cardiomyopathy	Dysarthria	Dysphagia	Wheelchair-bound	SDFS (Disability stage)	Total ADL	Total SARA	INAS count	SCAFI-8mTW	SCAFI-9HPT-D	SCAFI-9HPT-ND	SCAFI-PATA
1	+	R*	-	+	+	+	+	-	-	+	+	-	-	5	7	12	5	10.5	23	26	23.5
2	+	+	+	+	+	+	+	+	-	-	-	-	-	5	13	19.5	9	N/A	25	25	23
3	+	+	-	-	+	-	-	-	-	-	-	-	-	5	8	16	7	38.5	22.5	22.5	30
4	+	+	-	+	-	+	-	-	-	-	+	-	+	6	19	22	6	N/A	36.5	32.5	34
5	+	+	+	+	+	+	-	-	-	-	-	+	+	6	NK	NK	NK	NK	NK	NK	NK
6	+	-	+	-	+	+	-	-	-	-	-	-	-	5	6	11	4	N/A	24	24	36
7	+	+	+	+	+	+	+	-	-	-	-	-	-	4	NK	NK	NK	NK	NK	NK	NK
8	-	+	+	+	+	-	-	-	-	-	-	-	-	3	NK	NK	NK	NK	NK	NK	NK
9	+	+	+	+	-	+	+	-	-	+	+	-	-	3	4	8	4	13.5	21.5	22	21
10	+	-	-	+	+	-	-	-	+	+	-	+	-	4	7	7.5	4	12	26.5	23.5	22.5

*R=severe cogwheeling rigidity due to comorbid Parkinson's disease. Sample excluded from analysis of spasticity
ADL=Activities of daily living
SARA=Scale for the assessment and rating of ataxia
INAS=Inventory of non-ataxic symptoms
SCAFI=Spinocerebellar ataxia functional index
8mTW=9-metre timed walk
9HPT-D=9-hole peg test (dominant hand)
9HPT-ND=9-hole peg test (non-dominant hand)
NK=not known; N/A=not applicable

Table 32: Demographic details and clinical rating scales for compound heterozygotes and homozygous patients

	Compound Heterozygotes				Homozygous GAA+/+				Mann-Whitney U-test	
	Mean	SD	Range	*n*	Mean	SD	Range	*n*	Z	*p*†
AAO	14.1	2.5	10-19	10	13.8	9.8	1-55	159	-1.157	0.247
AAE	35.6	12.4	21-50	10	34.2	13.2	16-68	159	-0.543	0.587
DD	21.5	13.9	3-40	10	20.3	11.1	3-55	159	-0.163	0.870
SDFS	4.6	1.1	3-6	10	5.0	1.4	1-7	158	-1.655	0.098
Total ADL	9.5	5.5	4-19	6	15.6	8.3	1-35	154	-1.847	0.065
Total SARA	13.7	5.6	7.5-22	7	22.9	10.0	1.5-40	157	-2.349	0.019
INAS count	5.6	1.9	4-9	7	5.0	1.6	2-9	158	-0.519	0.604

AAO=Age at onset; AAE=age at examination; DD=disease duration; SD=standard deviation
SDFS=Spinocerebellar degeneration functional score
ADL=Activities of daily living
SARA=Scale for the assessment and rating of ataxia
INAS=Inventory of non-ataxic symptoms
SD=Standard deviation
†Bonferroni correction reduces significant *p* value to 0.05/7=0.0071

Table 33: Clinical features in compound heterozygotes and homozygous patients

Feature	Score	Compound Heterozygotes			Homozygous GAA+/+			Fisher's Exact
		n	%	*N*	*n*	%	*N*	*p*†
Paresis	INAS count[a]	9	90.0	10	122	77.2	158	0.693
Spasticity	INAS count[a]	7	77.7	9	76	48.1	158	0.099
Hyper-reflexia	INAS count[a]	6	60.0	10	7	4.4	158	<0.0005
Areflexia	INAS count[a]	8	80.0	10	149	94.3	158	0.130
Extensor plantars	INAS count[a]	8	80.0	10	98	62.0	158	0.327
Vibrational sensory loss	INAS count[a]	7	70.0	10	138	87.3	158	0.141
Broken pursuits	INAS score[b]	4	40.0	10	127	80.4	158	0.008
Square wave jerks	INAS score[b]	1	10.0	10	108	68.4	158	<0.0005
Diabetes	EFACTS Registry[c]	1	10.0	10	13	8.2	158	0.592
Cardio-myopathy	EFACTS Registry[c]	3	30.0	10	69	45.4	152	0.514
Dysarthria	ADL[d]	3	30.0	10	140	90.9	154	<0.0005
Dysphagia	ADL[d]	2	20.0	10	114	74.0	154	0.001
Wheelchair-bound	SDFS[e]	2	30.0	10	92	58.2	158	0.023

cont…

INAS=Inventory of non-ataxic symptoms
SARA=Scale for the assessment and rating of ataxia
EFACTS=European Friedreich's ataxia Consortium for Translational Studies
ADL=Activities of daily living
[a]Binary values taken from the calculation for the INAS count
[b]Taken directly from the INAS score
[c]From EFACTS Registry (various & cardio sections)
[d]Binary value derived from score of ≥1 on dysarthria & dysphagia questions of ADL
[e]Binary value derived from score of ≥6 on SDFS
†Bonferroni correction reduces significant *p* value to 0.05/13=0.0038

At the time of thesis production, the results of GAA expansion sizing were only available for 5 patients (those from phase 1 of the EFACTS cohort) of which four had the p.Gly130Val mutation and one the novel p.Arg165Asp mutation. Although there are too few data to make a meaningful analysis, there does still appear to be a trend to toward increased disease severity with the increased size of the larger of the two GAA expansions ('GAA2') as measured by earlier age at onset and greater total SARA (see Figure 52). This is likely to be significant as most of the point mutations were the same, eliminating this as a variable. In the case of compound heterozygotes the larger of the two expansions is used in this analysis, as the smaller (on the allele with the point mutation) is below the pathological limit of expansion. Interestingly, two of the patients (patients 1 & 4 with GAA1 sizes of 67 & 45 respectively) fall within the 'intermediate' zone (35-89 GAA repeats) between normal and pathologically expanded as used in the Neurogenetics laboratory of the NHNN. There may therefore be some small additional frataxin deficiency contributed by these alleles.

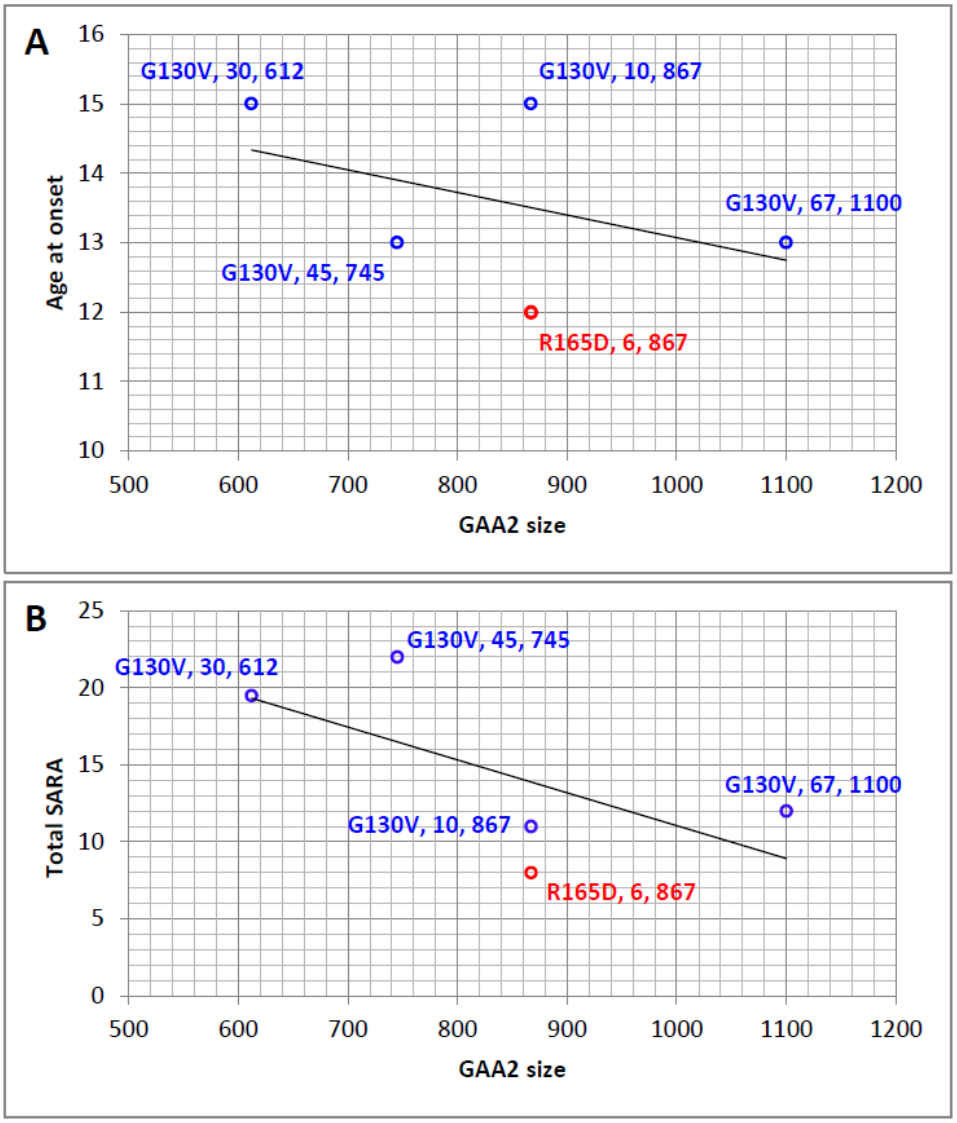


Figure 52: Correlation of GAA2 size with (A) age at onset and (B) total SARA in compound heterozygous FRDA patients

For each patient, the mutation (G130V in blue, R165D in red), GAA1 and GAA2 values are given

3.3 *FXN* Compound Heterozygotes with Large Deletions

3.3.1 Method

The database of the Neurogenetics Department of the NHNN was searched for requests for and positive results of *FXN* gene testing between 2003 and 2013. Positive results included patients with two GAA expansions, those with one GAA expansion and those with point mutations (search undertaken by Dr Mary Sweeney & Dr Robyn Labrum, Department of Neurogenetics, NHNN). Testing for GAA

expansions was undertaken in the Neurogenetics Laboratory of the NHNN using previously described techniques (Campuzano et al. 1996, Potdar & Raghu 2013, Warner *et al.* 1996). This involves triplet-primed PCR followed by fragment size analysis by capillary electrophoresis (ABI-3730 fragment analyser, Applied Biosystems), as well as long-range PCR using the GAA-F and GAA-R primers described by Campuzano and colleagues (GAA-F: 6-FAM-5'-GGG ATT GGT TGC CAG TGC TTA AAA GTT AG-3'; GAA-R: 5'-GAT CTA AGG ACC ATC ATG GCC ACA CTT GCC-3') . Expanded sequences were defined as having ≥90 GAA repeats. Sequencing for point mutations was undertaken in the Merseyside and Cheshire Regional Molecular Genetics Laboratory, Liverpool Women's Hospital, Liverpool, UK using previously published techniques (Pandolfo 2006) as described in 3.2.1 above.

Those individuals with an appropriate clinical history and either two GAA expansions, or one GAA expansion plus a pathogenic mutation were deemed to have FRDA either because of a homozygous or compound heterozygous mutation respectively. Clinical details were sought for all patients reported as having one GAA expansion. Stored DNA was requested for all patients having one GAA expansion and clinical symptoms (as the search revealed asymptomatic individuals with one GAA expansion, that is to say asymptomatic carriers). DNA is extracted in the accredited Neurogenetics laboratory of the NHNN using standard procedures and stored indefinitely at -70°C. The internal policy of the Department is only to subject individuals to mutational analysis if a single GAA expansion has already been found, and so the search revealed no individuals with homozygous *FXN* point mutations.

Large deletions were detected using the multiplex ligation-dependent probe amplification (MLPA) technique which has been used previously to detect exonic *FXN* deletions (Deutsch et al. 2010, Anheim et al. 2012, van den Ouweland et al. 2012). The General Protocol issued by the manufacturer was followed (MRC-Holland, Amsterdam, Netherlands; version MDP-v002, 23.1.2012). The commercially available SALSA MLPA P136-B2 Recessive Ataxias Probemix was used (MRC-Holland, Amsterdam, Netherlands; lot 0511). This contains probes against the five coding exons in the *FXN* gene (see Table 34). There is no probe for the non-

coding exon 6. The probemix also contains probes against the nine exons of the *APTX* gene and the 26 exons of the *SETX* gene. Thus, this probemix will incidentally detect exonic deletions in these genes. Mutations in *APTX* cause ataxia with oculomotor apraxia type 1, and those in *SETX* cause ataxia with oculomotor apraxia type 2.

The probemix also contains nine reference probes in exons of unrelated genes on different chromosomes, which provide quality control data. The reference probes bind to the following sites: *KDR* (4q12, exon 14), *IL4* (5q31.1, exon 1), *GHRHR* (7p14.3, exon 2), *KCNQ3* (8q24.22, exon 2), *CHEK1* (11q24.2, exon 8), *AMN* (14q32.32, exon 3), *SPG11* (15q21.1, exon 11), *COPS3* (17p11.2, exon 12) and *SMARCB1* (22q11.23, exon 7). Patients are presumed to have no deletions in these exons, or point mutations or SNPs at the site of probe binding. If any of these are present, this becomes apparent at the data analysis stage.

The probemix also contains internal quality control fragments (the 'QDX2 control fragment set') (see Table 35). This includes a 92nt control probe which behaves similarly to other MLPA probes and is used by the software for comparison to other control fragments. There are four 'Q-fragments' which are oligonucleotides containing both PCR primer sequences in a single molecule and are therefore not dependent on target DNA to be amplified during the PCR. They are present in extremely low concentrations so that if sufficient target DNA is used, they are out-competed by the amplicons of the MLPA probes. If the height of these peaks is greater than one third that of the 92nt control probe, this indicates that insufficient DNA was used or the ligation reaction failed. There are two 'D-fragments' which detect sequences within CpG islands which are known to be difficult to denature. If the height of these peaks is less than 40% that of the 92nt control probe, this indicates that DNA denaturation was incomplete resulting in unreliable results for probes detecting sequences in or within 5kb of CpG islands. These are typically near mRNA transcription start locations and so most commonly affect exon 1 probes. There are also probes detecting sequences within the Q and Y chromosomes allowing identification of the gender of the sample and hence gross identification of

incorrect sample use. The complete list of probes by fragment size including control fragments is given in Table 36 from the MRC-Holland Product Description.

Table 34: Probes in MHC-Holland SALSA MLPA P316-B2 Recessive Ataxias probemix

FXN Exon	SALSA MLPA Probe	Length (nt)	Ligation Site[a]	Start-End[a]	Distance to next Probe	Left (5') Probe-Right (3') Probe
1	10891-L11561	234	43nt before exon 1	70840233-70840293	10.9 kb	5':CAGCAAGACAGCAGCTCCCAAGT 3': TCCTCCTGTTTAGAATTTTAGAAGCGGCGGGCCACCAG
2	10893-L11563	208	463-464	70851166-70851235	6.7 kb	5':CAGAGTGTCTATTTGATGAATTTGAGGAAATCT 3': GGAACTTTGGGCCACCCAGGGTAAGATAAAACACCTT
3	10894-L13901	258	517-518	70857878-70857950	11.9 kb	5':CTCTCTAGATGAGACCACCTATGAAAGACTAGCA 3': GAGGAAACGCTGGACTCTTTAGCAGAGTTTTTTGAAGACCT
4	14129-L15733	472	24nt after exon 4	70869764-70869842	8.3 kb	5':CCATCCAGGTATGTAGGTATGTTCAGAAGTCA 3': ACATATGTAATTCTTAAAGACTTCCGAAATGTGACATTGTGGACCAT
5	10895-L11565	154	1448-1449[b]	70878073-70878130	-	5':TGGCCTGCACTGGGTTGTCCA 3': GGGAGACCTAGTGCTGTTTCTCCCACATATTCACATA

Table adapted from MHC-Holland Product Descriptions v8 (7.3.12) & v11 (10.12.15)
[a]NM_001161706.1 [b]NM_0001444.4

Table 35: QDX2 control fragments in MHC-Holland SALSA MLPA P316-B2 Recessive Ataxias probemix

Length (nt)	Name	Gene	Chromosomal Location
64	64nt Q-fragment	-	-
70	70nt Q-fragment	-	-
76	76nt Q-fragment	-	-
82	82nt Q-fragment	-	-
88	88nt D-fragment	*CARM1*	19p13.2
92	92nt control fragment	*IL1B*	2q13
96	96nt D-fragment	*JPH3*	16q24.2
100	100nt X-fragment	*AMOT*	Xq23
105	105nt Y-fragment	*UTY*	Yq11.221

Table adapted from MHC-Holland Product Description v8 (7.3.12)

Table 36: Complete list of probes by fragment size in MHC-Holland SALSA MLPA P316-B2 Recessive Ataxias probemix

Length (nt)	SALSA MLPA probe	Chromosomal position			
		reference	FXN	SETX	APTX
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA				
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation				
100	X-fragment: Specific for the X chromosome				
105	Y-fragment: Specific for the Y chromosome				
127	Reference probe 00797-00093	5q31			
134	Reference probe 07905-L07641	7p14			
140	**SETX probe** 10900-L11569			**Exon 3**	
148	**APTX probe** 10862-L11532				**Exon 5b**
154	**FXN probe** 10895-L11565		**Exon 5**		
160	**SETX probe** 10898-L14358			**Exon 17**	
166	**SETX probe** 10904-L11573			**Exon 7**	
172	**SETX probe** 10922-L11591			**Exon 21**	
178	Reference probe 08292-L08106	22q11			
184	**APTX probe** 10890-L11560				**Exon 9a**
189	**SETX probe** 10908-L11577			**Exon 11**	
193	**APTX probe** 10860-L11530				**Exon 3**
198	**SETX probe** 10919-L14357			**Exon 19**	
202	**SETX probe** 10912-L14356			**Exon 14**	
208	**FXN probe** 10893-L11563		**Exon 2**		
214	**APTX probe** 10888-L11558				**Exon 7**
219	**SETX probe** 10905-L11574			**Exon 8**	
226	**SETX probe** 10902-L14355			**Exon 5**	
229	**APTX probe** 10889-L11559				**Exon 8**
234	**FXN probe** 10891-L11561		**Exon 1**		
239	**SETX probe** 10914-L13898			**Exon 15**	
246	**SETX probe** 10925-L14354			**Exon 23**	
254	**SETX probe** 13128-L14348			**Exon 13**	
258	**FXN probe** 10894-L13901		**Exon 3**		
265	**SETX probe** 10918-L11587			**Exon 18**	
272	**SETX probe** 10926-L11595			**Exon 24**	
278	Reference probe 09361-L14546	17p11			
285	**SETX probe** 10897-L13900			**Exon 1**	
290	**SETX probe** 12748-L14353			**Exon 4**	
301	**SETX probe** 10921-L11590			**Exon 20**	
310	**APTX probe** 10863-L11533				**Exon 6**
319	**SETX probe** 10910-L11579			**Exon 12**	
328	**APTX probe** 10857-L11527				**Exon 1a**
337	**SETX probe** 10915-L11584			**Exon 16**	
346	Reference probe 08024-L07805	11q24			
355	**SETX probe** 10927-L14352			**Exon 25**	
364	**APTX probe** 10861-L14351				**Exon 4a**
373	**SETX probe** 10907-L11576			**Exon 10**	
382	Reference probe 06594-L06152	8q24			
391	**SETX probe** 12776-L11572			**Exon 6**	
400	**SETX probe** 12777-L11598			**Exon 26**	
409	**SETX probe** 12747-L13841			**Exon 2**	
417 *	**APTX probe** 14088-L15687				**Exon 2**
427	**SETX probe** 13129-L14349			**Exon 22**	
436	Reference probe 10304-L10817	14q32			
445	Reference probe 12758-L13874	4q12			
454 *	**APTX probe** 14089-L15688				**Exon 2**
463 *	**SETX probe** 14128-L15732			**Exon 9**	
472 *	**FXN probe** 14129-L15733		**Exon 4A**		
481 *	Reference probe 09772-L10187	15q21			

Table from MHC-Holland Product Description v8 (7.3.12). Note, fragment 472 was subsequently corrected to FXN exon 4 in later versions.

DNA quality and concentration were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). At least 150µg of genomic DNA was used per sample. For each patient, 5µl DNA solution in tris-EDTA buffer (TE) was denatured at 98°C for 5 minutes and cooled to 25°C on a thermocycler (Applied Biosystems Gene Amp PCR System 9700, Thermo Fisher Scientific, Waltham, MA, USA). A hybridization mastermix was prepared containing 1.5µl MLPA buffer and 1.5µl of probemix for each reaction. 3µl of the mastermix was added to each denatured DNA sample. The samples were hybridized by incubating for 1 minute at 95°C, then 16-20 hours at 60°C of the thermocycler. On day 2, a ligase mastermix was prepared containing 25µl distilled water, 3µl ligase buffer A, 3µl ligase buffer B and 1µl ligase 65 enzyme for each sample. The thermocycler temperature was lowered to 54°C. 32µl of ligase mastermix was added to each hybridized sample. The samples were ligated by incubating at 54°C for 15 minutes, and the enzyme inactivated by heating to 98°C for 5 minutes before cooling to 20°C.

The 'one-tube PCR' protocol was followed. A polymerase mastermix was prepared containing 7.5µl distilled water, 2µl carboxyfluoroscein-labelled (6-FAM) SALSA PCR primer and 0.5µl SALSA polymerase for each sample. 10µl of polymerase mastermix was added to each sample and the PCR program followed (35 cycles of 95°C for 30s, 60°C for 30s and 72°C for 60s). The samples were then held at 72°C for a further 20 minutes and then cooled to 15°C. The overall thermocycler program is given in Table 37.

On day 3, fragment analysis by capillary electrophoresis was undertaken using an Applied Biosystems ABI-3730 fragment analyzer (Thermo Fisher Scientific). 0.2µl GeneScan 500 LIZ dye size standard (Applied Biosystems) and 9µl Hi-Di formamide (Applied Biosystems) were added to 1µl of each PCR product. The samples were heated to 80°C for 2 minutes on the thermocycler, then cooled rapidly on ice for 3 minutes before transferring to the fragment analyzer. The data were analyzed using Coffalyser software (MRC-Holland) and GeneMarker v2.2.0 (Soft Genetics, PA, USA) which generates an EDQ for each probe.

Table 37: Thermocycler program for MLPA reaction

Stage	Temp	Time	
DNA denaturation	98°C	5 minutes	
	25°C	pause	
Hybridization reaction	95°C	1 minute	
	60°C	pause	
Ligation reaction	54°C	pause	
	54°C	15 minutes	
	98°C	5 minutes	
	20°C	pause	
PCR reaction	95°C	30 seconds	
	60°C	30 seconds	35 cycles
	72°C	60 seconds	
	72°C	20 minutes	
	15°C	pause	

Table adapted from MHC-Holland Product Description v8 (7.3.12)

3.3.2 Results

1768 genetic tests for FRDA were undertaken at the NHNN between 2003 and 2013. 196 cases (11.1%) showed homozygous GAA expansions in the *FXN* gene. Forty carried a single GAA expansion. Of these, eleven were found to have pathological point mutations on sequencing, making the total diagnosed cases of FRDA 207 (11.7% of tests requested). Thus 5.3% of the diagnosed cases of FRDA processed by the NHNN Neurogenetics laboratory were compound heterozygotes. Of the remaining 29 cases bearing a single pathological expansion, it was found on inspection of the clinical records that nine were asymptomatic carriers and had been referred either for predictive clinical testing and partner testing to inform reproductive decisions. Twenty cases were therefore suitable for the MLPA study. No DNA was available for 2 cases leaving 18 cases from NHNN to be subjected to MPLA analysis.

Figure 58 in the Discussion below shows the flow of these 18 NHNN patients through the MLPA study and the pathway by which patients were identified from the NHNN Neurogenetics database as having a single pathological GAA expansion and symptoms compatible with FRDA. Analysis of these patients' results therefore provides evidence as to whether exonic deletions are common in patients who present with ataxia and have a single GAA expansion and no associated point

mutation, and so whether testing for exonic deletions is indicated in such patients. The implications of this for a diagnostic neurogenetics laboratory are explored in the Discussion.

In addition to the above 18 patients from the NHNN database, a number of other patients from the EFACTS project were also studied. Table 38 shows all the patients in the study with the EDQ results for each *FXN* exon. Samples 1 to 18 are the 18 NHNN patients described above. Samples 19 and 22 are duplicate runs of the the proband patient D described in 3.1.2.1 above and 3.3.2.1 below. Sample 20 is a negative control used by the NHNN laboratory who is known clinically not to have FRDA and who does not have pathological GAA expansions. The individual in fact has dopa-responsive dystonia with a mutation in the GTP cyclohydrolase 1 gene on chromosome 14q22.1-q22.2. This sample was set as the control sample for the study which the software uses to calculate the EDQ for the disease exons. Sample 21 is a 'no DNA' control containing TE buffer in place of a DNA sample to test for contamination of any of the reaction constituents by extraneous DNA. Samples 23 to 26 are EFACTS patients who were reported by the laboratory of the Université Libre de Bruxelles as having identically sized GAA expansions (1200 repeats for sample 23, 1167 for sample 24, 1000 for sample 25, 834 for sample 26). Their test result is therefore subject to the same potential misattribution as that of patient D as the finding of a single band on triplet-primed PCR does not exclude the possibility of the patient having a single pathological GAA expansion as well as an exonic deletion.

The MLPA reaction failed for samples 2 and 4. These have subsequently been repeated by Mr Alex Browne (PhD student) with the same result. The TE control also failed showing that there was no DNA contamination of the reagents. An EDQ result of 1±0.25 indicates no evidence of exon deletion or duplication. A result of 0.5±0.25 indicates heterozygous deletion at that exon, whilst a result of 0±0.25 indicates a homozygous deletion. A result of 1.5±0.25 indicates heterozygous duplication and 2±0.25 homozygous duplication. Theoretically, the higher gene multiplications such as triplications could further modify this result although these have not hitherto been described in FRDA. The EDQs for the five *FXN* exons in the

16 GAA +/- cases for which MLPA results were available, were all within 1±0.25 indicating no evidence of exon deletion or duplication at each of the probe sites. The mean±SD EDQ for these samples was 1.004±0.046 (range 0.886-1.133). The EDQs for all the remaining samples inlcuding the two samples from patient D and the four EFACTS samples, were also all within the range 1±0.25. All the cases together (including controls) had a mean±SD EDQ of 1.004±0.043 (range 0.886-1.151). The results are shown in Table 38. Figure 53 as an example shows the signal intensity peaks for each of the probes for sample 22 (patient D) in blue and the GAA -/- control (sample 20) in red, with the five *FXN* exon probes individually labelled. The ratio of the blue to red peaks generates the EDQ; it can be seen that they are all of similar height, hence the ratios are all aproximate to unity. Figure 54 shows the peak ratios (EDQs) for the same sample with the disease probes in green and reference probes in blue. The five *FXN* gene probes are again highlighted. All values are within the upper and lower limits of normal, indicating no evidence for deletions or duplications of any exon of the *FXN* gene. As an incidental finding, no evidence was also found in any of the samples of *APTX* or *SETX* deletions or duplications (data not presented).

Table 38: *FXN* exon dosage quotients (EDQ) for patients in MLPA study

Sam-ple	Pre-test status	EDQ Exon 1	EDQ Exon 2	EDQ Exon 3	EDQ Exon 4	EDQ Exon 5
1	GAA +/-	1.033	0.925	1.065	1.080	0.887
2	GAA +/-	-	-	-	-	-
3	GAA +/-	1.050	1.032	1.014	1.040	1.007
4	GAA +/-	-	-	-	-	-
5	GAA +/-	1.126	0.971	1.061	1.065	0.886
6	GAA +/-	1.040	0.994	0.981	1.010	0.988
7	GAA +/-	1.069	1.016	1.049	1.133	0.942
8	GAA +/-	1.091	1.024	1.005	1.011	0.988
9	GAA +/-	1.041	0.913	0.982	0.956	0.909
10	GAA +/-	1.024	0.996	1.009	0.982	0.972
11	GAA +/-	0.992	1.004	0.989	0.965	0.968
12	GAA +/-	1.036	1.023	0.981	0.943	0.993
13	GAA +/-	1.056	1.017	1.020	0.965	0.988
14	GAA +/-	1.017	1.028	0.990	1.025	0.996
15	GAA +/-	1.013	1.035	0.991	1.031	0.993
16	GAA +/-	1.028	1.010	0.970	1.003	0.982
17	GAA +/-	1.009	0.989	0.976	0.930	0.983
18	GAA +/-	1.021	1.048	1.012	0.946	1.008
19	Patient D	1.055	1.023	0.999	0.981	0.988

20	GAA -/-	1.000	1.000	1.000	1.000	1.000
21	TE control	-	-	-	-	-
22	Patient D	1.071	1.030	1.000	0.993	0.988
23	GAA +/+	1.030	0.983	0.962	0.985	0.975
24	GAA +/+	1.151	0.980	1.015	0.944	0.959
25	GAA +/+	1.012	1.009	1.014	0.981	0.979
26	GAA +/+	1.061	1.004	1.031	0.960	0.962
Mean ±SD	All GAA +/-	1.040±0.033	1.002±0.038	1.006±0.030	1.005±0.056	0.968±0.040
Range		0.992-1.126	0.913-1.048	0.970-1.065	0.930-1.133	0.886-1.008
Mean ±SD	All values	1.044±0.038	1.002±0.033	1.005±0.027	0.997±0.049	0.971±0.034
Range		0.992-1.151	0.913-1.048	0.962-1.065	0.930-1.133	0.886-1.008

GAA +/- one GAA expansion (ie symptomatic carrier)
GAA -/- no GAA expansions (ie negative control)
GAA +/+ two GAA expansions (ie known FRDA patient reported as having two identically sized expansions)
TE=Tris-EDTA buffer (ie no DNA control)
SD=standard deviation

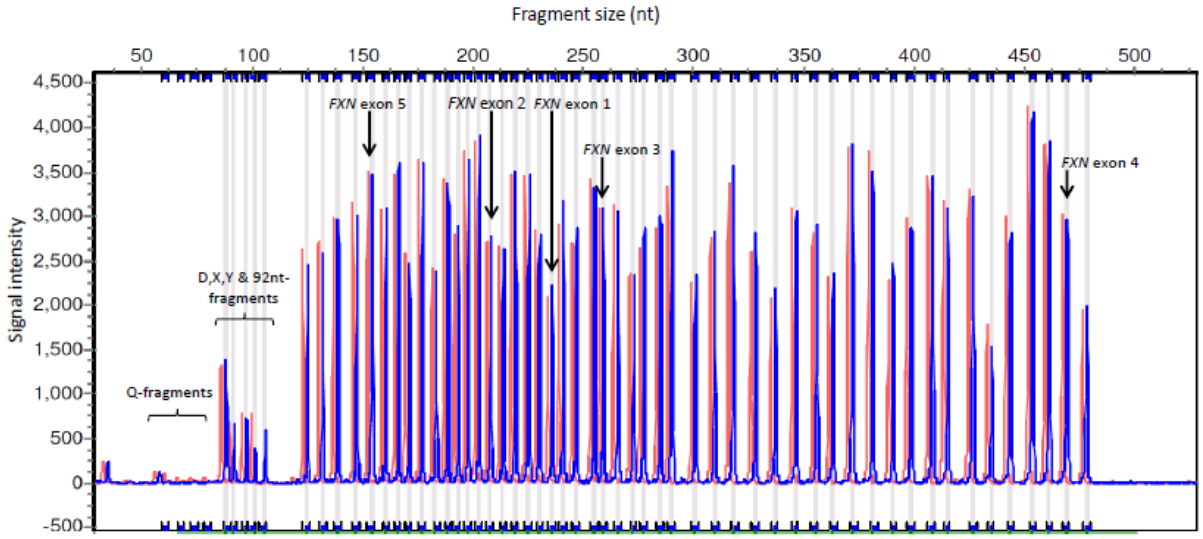


Figure 53: Peak height comparison chart for sample 22 (patient D)

Chart generated by GeneMarker software. Blue peaks=patient samples; red peaks=control samples. 64-82 peaks=Q-fragments; 88-105 peaks=D,X,Y & 92bt control fragments; 128-481 peaks=*FXN, SETX, APTX* & 9 control genes including *FXN* peaks at 154, 208, 234, 258 & 472.

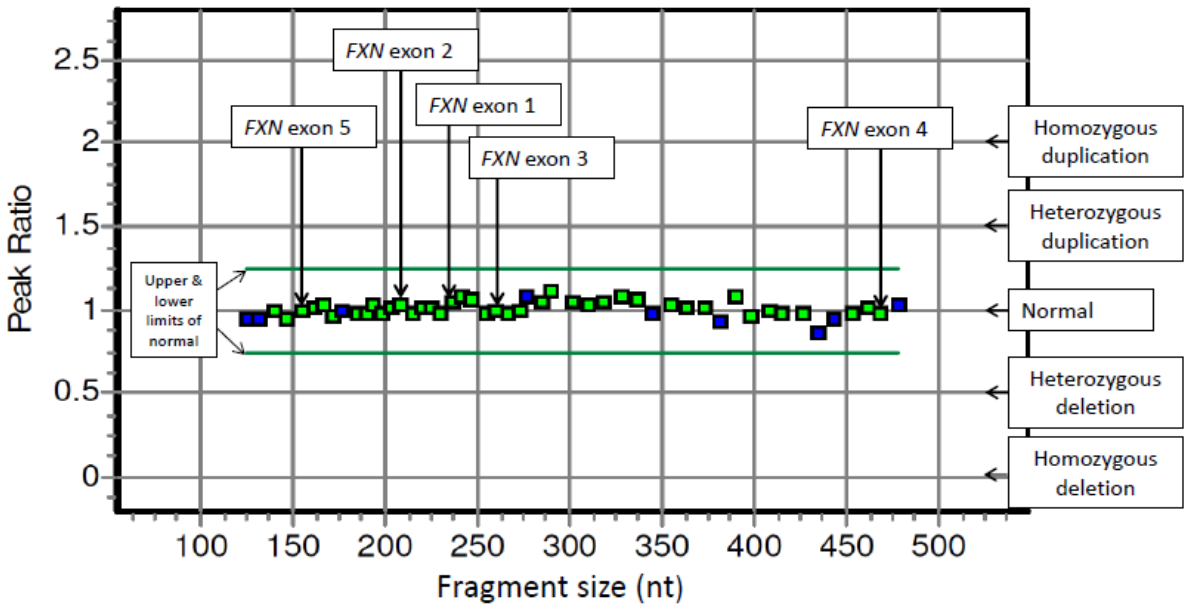


Figure 54: Peak ratios (EDQ) for sample 22 (patient D)

Chart generated by GeneMarker software. Peak ratio (EDQ) for disease (green) and reference (blue) probes. The five *FXN* gene probes are highlighted (154, 208, 234, 258 & 472nt). The remainder are for *SETX* and *APTX*. All values are within the upper and lower limits of normal, indicating no evidence for deletions or duplications of any *FXN* exon.

3.3.2.1 Case History: Patient D

The proband (patient D) was a right-handed Caucasian male who was 30 years old at the baseline assessment. His first symptoms were of gait instability at one-and-a-half years of age. He walked late at 18 months and was always very tired as a child and had to be carried. He first noticed significant falls at age 9 and started using an aid to walking intermittently at age 16, and then permanently at age 18. He started using a wheelchair at age 23 and by the baseline assessment was unable to stand or walk. FRDA was diagnosed at age 20. He does not have diabetes but was diagnosed with cardiomyopathy at age 26. ECG at age 33 showed sinus rhythm with voltage evidence of left ventricular hypertrophy. Echocardiogram at the same time showed IVSd of 13mm, LVPWd of 11mm and left ventricular ejection fraction of 46%. He has moderate spinal scoliosis which has never required surgical correction. He has moderate bilateral pes cavus and mared bilateral talipes varus. Both feet were operated on at age 26. He has some hearing impairment but does not use a hearing aid. Other medical problems include myopia, gastro-oesophageal reflux disease and psoriasis.

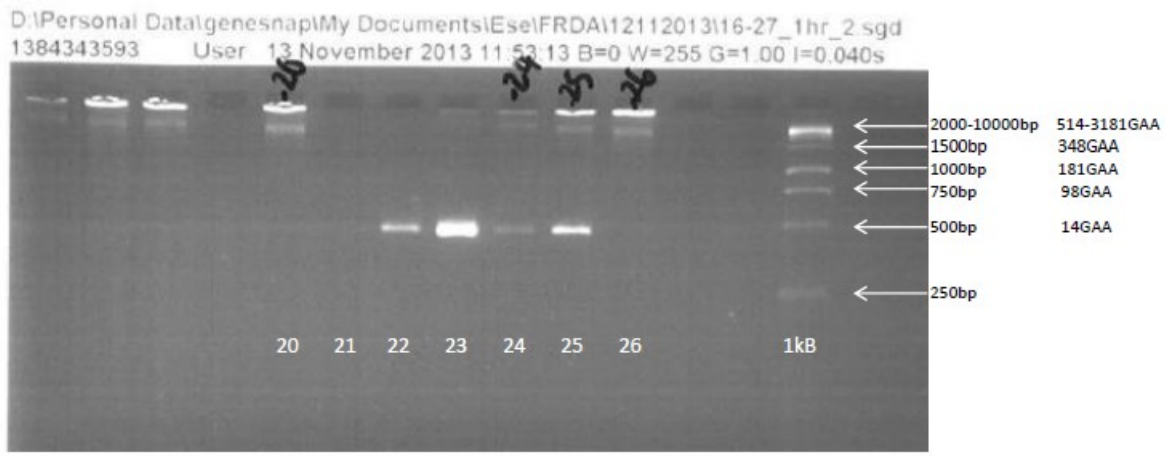
Total SARA was 33/40 (gait 8, stance 6, sitting 4, speech 3, finger chase 2, nose-finger 3, alternating hand movements 3, heel-shin 4). The gait, stane, sitting and heel-shin subscores represent the maximal for these fields. The SDFS was 6. Total ADL was 25/36 (speech 3, swallowing 2, use of cutlery 2, dressing 3, washing 3, falls 4, walking 4, sitting 4, bladder function 0). The walking, falls and sitting subscores are therefore at their maximal values. On the SCAFI, he was unable to undertake the 8mTW. He managed the 9HPT using his dominant hand in 232 and 202s, but was unable to complete the task using his non-dominant hand. His PATA score was 14 and 13 words in 10s. The INAS count was 5/16 gaining points for areflexia, extensor plantar reflexes, spasticity, paresis and vibrational sensory loss.

Neurological examination showed no facial weakness or sensory loss. There was no lingual atrophy but moderate lingual spasticity resulting in marked dysarthria. There was no ptosis or ophthalmoparesis. There were saccadic intrusions into pursuit eye movements with bilateral horizontal gaze-evoked nystagmus and hypometric saccades but no square wave jerks. There was mild (4+/5) proximal UL weakness with mild UL spasticity but no atrophy. There was distal UL pin prick and proprioceptive sensory loss but no vibrational sensory loss. There was areflexia throughout the upper and lower limbs. In the LLs, there was severe weakness (0/5) throughout with no spasticity or atrophy. There was marked distal pin prick, proprioceptive and vibrational sensory loss. The plantar reflexes were plantar bilaterally. There was no evidence of myoclonus, chorea, dystonia or parkinsonism.

The total SARA at BL, FU1 and FU2 was 33, 31 and 34 respectively. The total ADL was 25, 28 and 26 respectively. The INAS count was 5, 5 and 4 respectively. The SDFS remained 6 throughout. The only complete part of the SCAFI was the PATA test. The mean scores were 13.5, 12.5 and 16 respectively.

Long-range PCR in the NHNN Neurogenetic laboratory confirmed the findings of the Exeter laboratory (see Figure 55). Patient D is in lane 20 showing a single expanded band sized at between 514 and 3181 GAA repeats. Lanes 22 and 23 show two unaffected individuals with no GAA expansions. Lanes 24 and 25 show

two heterozygous carriers with one GAA expanded and one non-expanded allele, Lane 26 shows a known FRDA patient with two GAA expansions.



Lane 20=Patient D. Lane 21='no DNA control'. Lanes 22/23=Negative (GAA-/-) controls.
Lanes 24/25=Carriers (GAA+/-). Lane 26=Positive control (GAA+/+).
1kB size ladder (Promega, Madison, WI, USA) with equivalent GAA sizes.
Gel courtesy of Dr Robyn Labrum & Dr Ese Mudanohwo.

Figure 55: Long-range FXN PCR for patient D

There is a strong family history of neurological problems. The patient has a brother and a sister neither of whom has balance, coordination or gait problems, but his brother has a complex cranial tic involving blepharospasm and versive head movements. Neither was examined as part of the study. His mother was examined as part of the study. She was born to a normal birth without consanguinuity. She walked at 14 months but always had problems with riding a bicycle and playing sports, and had difficulty walking in the dark without assistance. She continued to have balance problems as an adult and was extremely sensitive to alcohol which caused unsteadiness and slurred speech. At around age 30-40, she started to notice sensory loss and tingling in the hands on waking, and problems with manual dexterity. Previous neurophysiological investigations were said to have shown a predominantly sensory axonal neuropathy. She has hearing difficulty in conditions of background noise.

On examination, the only sensory loss was decreased pin prick sensation on the lateral borders of the feet and shins. The reflexes were all present and normal. The plantar reflexes were mute. Cranial nerve examination including the eye movements was normal. The total SARA was 9 (gait 2, stance 3, sitting 1, speech 0,

finger chase 1, nose-finger 0, alternating hand movements 1, heel-shin 1) although it was felt that many aspects of the examination were dominated by distractible functional overlay. A routine panel of blood tests showed no cause of neuropathy. Electromyography and nerve conduction studies in this institution showed no evidence of a generalized neuropathy but there was evidence of median neuropathies at the wrist, consistent with bilateral carpal tunnel syndrome. Upper and lower limb sensory evoked potentials were within normal limits. MR imaging showed normal intracranial appearances with no regional or generalized volume loss. There were mild degenerative changes in the cervical and lumbar spine with moderate intervertebral foraminal narrowing on the left at C3/4. The vertebral canal was capacious and the spinal cord of normal calibre and signal intensity throughout. A next generation sequencing panel of genes for inherited neuropathies (Charcot-Marie-Tooth syndromes type 1 & 2) showed only two rare variants in the genes *TRPV4* and *MARS* which were not predicted to be pathological.

The sister of the patient's mother also had problems with balance although had had a horse-riding accident in her 30s. Both of her daughters also had problems with balance. The father of the patient's mother also had problems riding a bicycle and his brother walked with a stick and had speech problems from early in life. None of these individuals was examined as part of the study.

3.4 Discussion

This Chapter provides a comprehensive clinical and genetic assessment of two rarer causes of FRDA, namely compound heterozygotes involving either point mutations or large deletions.

3.4.1 *FXN* Compound Heterozygotes with Point Mutations

Compound heterozygous FRDA patients with four different *FXN* point mutations were identified as part of this study. The mechanism of damage for the c.2T>C/p.Met1Thr and c.357_378dup22/p.Phe127fsX mutations is clear, given that they either induce incorrect translational initiation or introduce premature

termination. The second mutation has not previously been published and represents the first case described of a duplication in the *FXN* gene.

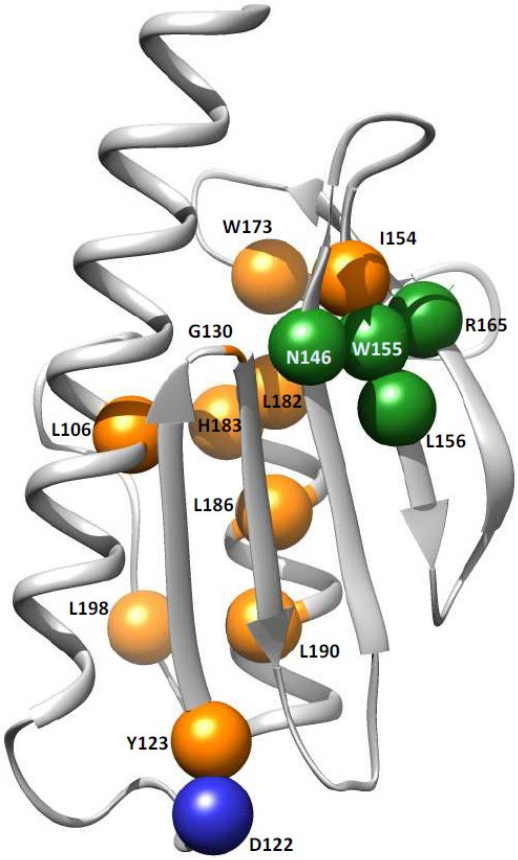


Figure 56: Tertiary structure of human frataxin showing selected mutated residues

Green spheres show IscU-binding residues including Arg165 (R165). Blue sphere shows ferrochelatase-binding residue. Gold spheres show non-binding residues including Gly130 (G130). Coils are α-helices. Arrows are the β-strands. Figure from Galea *et al.* (2015)

The Arg165 residue lies within the β_5 strand of the β-sheet which binds the iron-sulphur cluster scaffold protein, IscU (see Figure 56). The p.Arg165Asp mutation involves a change from an arginine residue whose side chain is long and cationic under physiological conditions ($-CH_2CH_2CH_2NHCHNHNH_3^+$) to an aspartate residue whose side chain is short and anionic ($-CH_2COO^-$). This change is predicted to have little effect on protein stability but to affect interactions with IscU and so disrupt ISC biosynthesis (Galea *et al.* 2015). Studies of the interaction of the bacterial orthologue of frataxin, CyaY, with IscU and IscS suggest that a ternary complex is formed, with IscU interacting with a series of conserved arginine residues on the β-sheet (Prischi *et al.* 2010). This mutation has not been described previously but

previous descriptions of mutations involving different nucleotide changes resulting in different amino acid changes at the Arg165 residue make this a mutational hot-spot.

The Gly130 residue lies within a tight turn formed by residues Gly128, Ser129 and Gly130 between strands β_1 and β_2 of the β-sheet (Correia *et al.* 2008) (See Figure 56). Introduction of the larger valine residue introduces local strain at that point. The backbone carbonyl oxygen atom of the Gly130 residue also forms a hydrogen bond with the backbone amide hydrogen atom of the Lys147 residue in the neighbouring β_3 strand which helps preserve the integrity of the β-sheet (see Figure 57). Previous thermodynamic studies confirm that the p.Gly130Val variant has lower stability than wild-type protein which may relate to these effects (Correia et al. 2008). Modelling predicts that substitution of valine for glycine at residue 130 results in the loss of this hydrogen bond. This may be because of a steric clash between the larger aliphatic valine side chain [$-CH(CH_3)_2$] and that of lysine [$-CH_2CH_2CH_2CH_2NH_3^+$]. The lysine side chain amino group forms a strong electrostatic interaction with the side chain carboxyl moiety [$-CH_2CH_2COO^-$] of the Glu96 residue within the α_1 helix (see Figure 57). This may prevent the Lys147 residue from adopting a different conformation to accommodate the large aliphatic side chain of the p.Gly130Val variant (Galea *et al.* 2015). Taken together, these changes are likely to decrease frataxin stability *in vivo* and disrupt the binding of the β-sheet with the iron-sulphur cluster scaffold protein, IscU, and thus disturb ISC biogenesis. The Gly130Val mutation was one of the first *FXN* point mutations to be described. The results of this thesis suggest that it may be the commonest in the UK. There is evidence from haplotype analysis that affected individuals from as far afield as the USA, France and Australia may share a common founder (Delatycki *et al.* 1999). This could be tested in the UK cases.

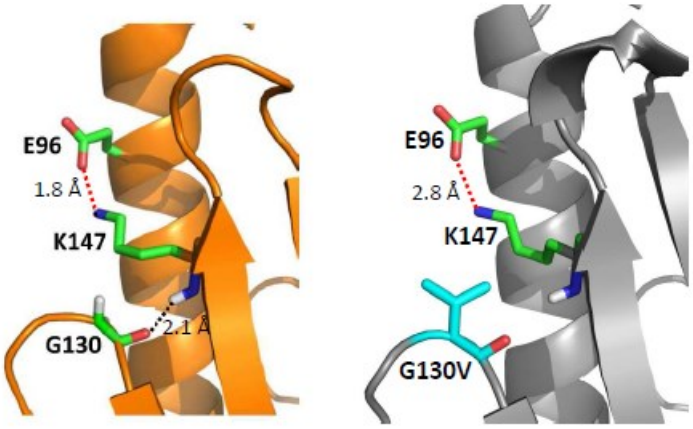


Figure 57: Effect of Gly130Val mutation on frataxin tertiary structure
Left panel shows wild-type protein structure with hydrogen bond between the Gly130 (G130) residue and the Lys147 (K147) residue and the electrostatic interaction with residue Glu96 (E96). Right panel shows the disruption of the hydrogen bond by the introduction of valine at residue 130 (G130V). Figure from Galea *et al.* (2015)

The clinical results of the patients studied within this thesis suggest that the existence of hyperreflexia, and lower frequency of square wave jerks, dysarthria, dysphagia and possibly broken pursuit eye movements and wheelchair-bound status are associated with the compound heterozygous state. Of the clinical rating scales, only the SARA came close to detecting a difference between the two groups suggesting a lesser degree of ataxia. This further confirms the greater utility of the SARA as a clinical rating scale in FRDA compared to other scales. There appears to be a residual effect of the size of the one expanded GAA repeat sequence on disease severity. These findings are consistent with previous reports that compound heterozygotes have an atypical milder phenotype with retained or brisk reflexes, absence of dysarthria and lower severity of ataxia (Cossée *et al.* 1999) with a residual effect of GAA2 size (De Castro *et al.* 2000, Gellera *et al.* 2007). Thus there is a suggestion that disease progression is slower. This thesis does not find, however, that age at onset was later amongst the compound heterozygotes. This may be because of the small number of cases studied and the high proportion of the mild p.Gly130Val missense mutation.

With this in mind, the cases described above which had adequate clinical information were pooled in a worldwide study incorporating cases from major

research centres in the USA, Australia and Europe which is due for publication in 2016 (Galea *et al*. 2015). This found 111 compound heterozygotes making it by far the largest study of FRDA compound heterozygotes yet published. The study found that patients with null mutations had significantly earlier onset of symptoms than homozygotes and compound heterozygotes with missense mutations, but there was no significant difference between homozygotes and those with missense mutations. Homozygotes were more likely to have cardiomyopathy than both null and missense mutants. Individuals with null mutations were 4.5 times more likely to have diabetes than homozygotes but there was no difference between homozygotes and missense mutants.

One intriguing question which neither this thesis nor the study of Galea resolves is why there has never been a case of a homozygous point mutant described. Homozygous null mutations would presumably be devastating if not lethal *in utero*. However, given the relatively mild clinical nature and relatively common incidence of the p.Gly130Val mutation, one might expect homozygous individuals to exist, particularly in regions in which consanguinuity is common. None has yet been described. In the UK, only symptomatic individuals with at least one pathological GAA expansion are tested for point mutations, which potentially makes the absence of discovery of homozygous point mutants a self-fulfilling prophecy. It may be that the alteration in function of the protein caused by the point mutations is sufficient to make homozygous point mutants inviable, in a way that simple deficiency of the otherwise normally functioning protein in individuals with homozygous pathological GAA expansions does not. Further work is warranted on this subject.

3.4.2 *FXN* Compound Heterozygotes with Large Deletions

3.4.2.1 Frequency of Large Deletions amongst FRDA Patients

The principal objective of the MLPA study was to assess the frequency of exonic FXN deletions in a large cohort of patients referred to a tertiary neurogenetics service with a clinical history compatible with FRDA. This is important as, in this situation, most neurogenetics laboratories currently look for the presence or absence of pathological GAA expansions by triplet-primed and long-range PCR. If

two expansions are found, a genetic diagnosis of FRDA is made. If one GAA expansion is found in a symptomatic individual, the DNA is subjected to sequence analysis for point mutations. This process therefore only allows the diagnosis of FRDA caused by homozygous GAA expansions, or compound heterozygous GAA expansions and point mutations. We wished to test the hypothesis that symptomatic individuals with one GAA expansion and no point mutation might also have an exonic deletion.

To that end, details of all the referrals to the specialist Neurogenetics laboratory at the NHNN for genetic testing for FRDA over a 10 year period were obtained. Figure 58 shows the results of genetic testing on these samples both in the NHNN laboratory and as part of this project. No exonic deletions were detected as part of the MLPA study, either from the NHNN or EFACTS patients. It is therefore reasonable to assume that the proportion of FRDA patients with an exonic deletion is exceedingly low, so low that none was detected amongst 10 years' referrals to a major tertiary referral centre for neurogenetics with a particular interest in the hereditary ataxias. It may be that none of these mutations is present in the UK population. None has so far been described. Of the eleven patients previously described in the medical literature, two were from the USA and nine from Western Europe (Germany, Netherlands, France and Norway). It therefore seems reasonable that this test is not routinely offered in the UK, although it might still be considered on a case-by-case basis if there is a very strong clinical suspicion of FRDA and a single pathological GAA expansion or point mutation is found.

Previously described cases with exonic *FXN* deletions have been identified because of the strong clinical suspicion of FRDA but inconclusive genetic results (van den Ouweland *et al.* 2012, Zühlke *et al.* 2004). The case described by Zühlke and co-workers was identified from a screen of fourteen patients, six of whom had a single pathological GAA expansion and all clinical features of ataxia. The two cases identified by Deutsch, Brigatti and others were derived from a screen of at least six patients but presumably taken from an extensive cohort, as the main research group involved (Children's Hospital of Philadelphia) is one of the principal centres of FRDA research in North America and the coordinating centre of a large multi-centre

longitudinal study of FRDA (the Collaborative Clinical Research Network in Friedreich's Ataxia) (Deutsch et al. 2010, Brigatti et al. 2012). The six patients described by Anheim and colleagues were identified by screening thirteen patients taken from an estimated 600 patients seen over twelve years, although the search does not appear to be systematic (Anheim *et al.* 2012). The most recently published study of FRDA in Norway aims systematically to identify every diagnosed case of FRDA in that country which is presumably possible because of the small population and limited number of centres involved. From a population of 5.1 million, 27 cases of FRDA with two GAA expansions were found, as well as one compound heterozygote with a point mutation and one with an exonic deletion (Wedding *et al.* 2015). The present thesis therefore includes the largest study to date which has systematically screened a large consecutive cohort of patients for exonic *FXN* deletions.

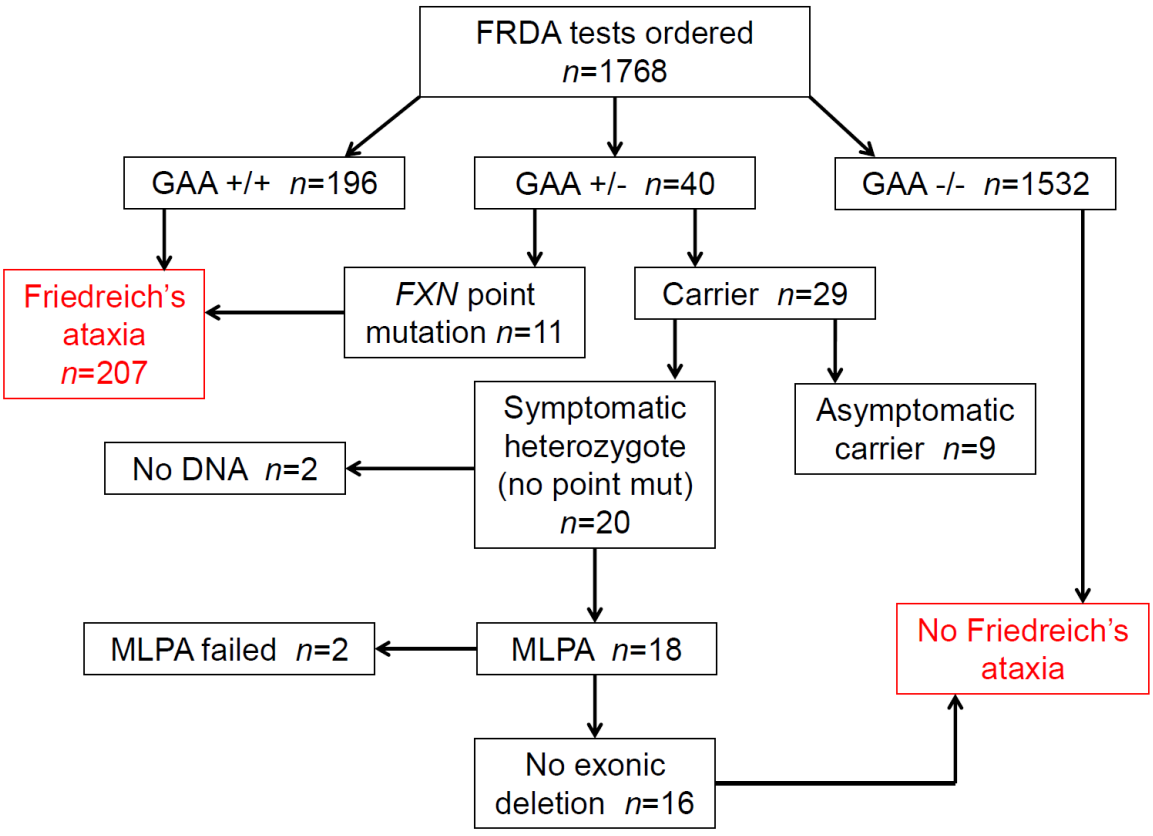


Figure 58: Flow diagram of patients in MLPA study
GAA +/+ two GAA expansions; GAA +/- one GAA expansion; GAA -/- no GAA expansions. MLPA=multiplex ligand-dependent probe amplification

This study also provides some interesting incidental epidemiological information. 207 positive diagnoses of FRDA were made over 10 years out of a total of 1768 referrals, giving a diagnostic rate of 11.7%. Eleven of those diagnoses were from compound heterozygous point mutations, making the proportion 5.3%. No systematic studies have been undertaken to determine the prevalence of *FXN* point mutations in the general population but Campuzano and colleagues found eleven patients out of 258 patients from two different cohorts to have point mutations (all compound heterozygotes), making a rate of 4.3%. They therefore reported GAA expansions as being present in approximately 98% of chromosomes studied (and not 98% of patients as is commonly reported in many academic papers) (Campuzano *et al.* 1996). Cossée and colleagues found 25 patients compound heterozygous for a point mutation out of 719 patients with a genetic diagnosis of FRDA, making a rate of 3.5% (Cossée et al. 1999). De Castro and colleagues found four patients with a point mutation out of 179 with a genetic diagnosis of FRDA, giving a rate of 2.2% (De Castro et al. 2000).

Twenty-nine GAA expansion carriers were found. Twenty were symptomatic and nine asymptomatic, making a carrier rate of between 1.1 and 1.6% or approximately 1/88 to 1/61. No systematic studies have been undertaken assessing the frequency of FRDA carrier status in the UK but Harding & Zilka estimated the rate in Great Britain at approximately 1/110 (Harding & Zilkha 1981). Other studies in Western Europe have estimated the carrier rate at 1/191 in the Torino region of North-western Italy (Leone *et al.* 1990), 1/85 in France (Cossée *et al.* 1997b), between 1/60 and 1/90 in Germany (Epplen *et al.* 1997) and 1/196 in Norway (Wedding et al. 2015). Filla and co-workers estimated the prevalence of FRDA in the Molise region of Southern Italy to be between 1/20,000 and 1/250,000 (Filla *et al.* 1992). Assuming the population is in Hardy-Weinberg equilibrium and there is no consanguinuity (both of which may not be true), these correspond to carrier rates of between 1/77 and 1/177. A further study in Cantabria, Spain found a prevalence rate of 1/21,277 which corresponds with a carrier rate of 1/73 (Polo *et al.* 1991). Of note, all of the studies published before 1996 rely on clinical rather than genetic diagnoses which introduces a further level of uncertainty. Whilst the present

sample may not be wholly representative of the UK population and no attempt was made for this to be a comprehensive epidemiological study, the results provide further information on this subject and are broadly in line with previous analogous studies.

3.4.2.2 Patient D

The rationale for studying patient D is given in the Introduction at 3.1.2.1. The method for the MLPA technique used to analyze patient D is given in Section 3.3.1 and the results in Section 3.3.2. Patient D's history is given in Section 3.3.2.1. The case of patient D is interesting as the most likely explanation of his genetic abnormality is a large deletion, and so he might represent the twelfth case so far described and the first in the UK. Unfortunately, this study does not determine the exact site of the genetic abnormality. There is no deletion of exons 1 and 2 detected by the MLPA technique. It is known that both the long-range PCR using two different sets of primers produced the same result of a single expanded band on the PCR gel. Triplet-primed PCR is uninformative as this cannot discern a single heterozygous expansion from two homozygous expansions. It is further known that long-range PCR using the primers described in Filla *et al.* (1996) in the patient's mother showed no expanded band. Figure 59 shows the locations of these primers and the MLPA probes relative to exons 1 and 2 and the GAA repeat sequence. The GAA repeat sequence is located 1.3kbp from the start of intron 1 which itself is 10.4kbp in length. The Filla and Campuzano primers bind within 150-200bp on either side of the GAA repeat sequence. The exon 1 probe binds in the first half of exon 1, 390bp from the exon 1-intron 1 splice site. The exon 2 probe binds in the second half of the much smaller exon 2, 45bp from the intron 1-exon 2 boundary and in fact overlaps the exon 2-intron 2 boundary.

Thus, a number of possibilities might have given rise to patient D's result. Genetically, he could have a small deletion encompassing either or both of the primer sets flanking the GAA repeat sequence. Indeed, any small sequence change preventing the binding of these primers could give rise to this result (akin to a SNP under a conventional primer), although it would have to be under the GAA-R/GAA-629R pair as the GAA-F/GAA-104F pair do not overlap. However, the patient does

have symptoms and so presumably exon 1 or 2, or both, is also involved. Alternatively, either or both of their splice sites could be involved. A large deletion seems the most likely explanation. This could extend from one of the primer sites toward exon 1 and encompass part of the 390bp of exon 1 downstream of its probe binding site, or toward exon 2 and include part of the 45bp of exon 2 upstream of its binding site, or indeed both. Further analysis of the deletion breakpoints is required.

Patient D has a severe clinical phenotype with very early symptom onset at age 1½ progressing to wheelchair dependency, cardiomyopathy, scoliosis and pes cavus. He does not however have diabetes. This is in keeping with previous observations that truncating mutations or those in the amino terminal half of the protein produce a more severe phenotype. Patient D has a GAA expansion of 785 repeats which would be expected to cause significant frataxin deficiency. A deletion of one or both of the first two exons would most likely result in no usable frataxin. Further work remains to determine the exact nature and extent of the mutation and other downstream effects such as frataxin protein and mRNA levels. Further clinical and genetic assessment is also warranted of other affected family members, the significance of which remains unclear.

Four additional cases were included in the MLPA study as a limited exploratory investigation of whether the EFACTS cohort contained other examples of patients reported as having equally sized expanded GAA sequences, but who in reality might have a single GAA expansion and a deletion encompassing the GAA repeat sequence or its associated primer binding sites. None of these cases showed exonic deletions. There are a further eleven such cases in the UK wing of the EFACTS cohort reported as having equal-sized GAA expansions who could be tested, and presumably many more in the whole European sample. However, the current study does not provide any additional examples of this phenomenon.

Nevertheless, the example of patient D is potentially significant as, if this phenomenon is more widespread than currently appreciated, although it is unlikely to lead to a *clinical* misdiagnosis in an affected individual, the *genetic* diagnosis

would be inaccurate. If disease-modifying medications become available which specifically interact with the GAA expansion or its related features such as epigenetic modifications, these may not be effective if the patient's disease is not associated with this pathological mechanism. It is also important in the interpretation of genetic tests of relatives being undertaken to provide reproductive advice. An asymptomatic carrier of this deletion might be falsely reassured that she could not pass on the genetic abnormality if her routine genetic test with a single normal-sized band were interpreted as providing evidence of two normal-sized alleles. This misinterpretation is potentially more common than that of expanded alleles, as *most* patients with unexpanded alleles only have a single small band on long-range PCR because the range of normal repeat sizes is much narrower than that of expanded repeat sequences.

The unusual nature of patient D's mutation was discovered effectively by accident as his mother was also tested as she had neurological symptoms. The parents of affected individuals are rarely tested as part of the diagnostic process for a variety of reasons. It adds expense and complexity to the diagnostic process. They may simply not be present, available or indeed alive. There are ethical considerations in the routine testing of parents - particularly fathers - because of the possibility of inadvertently revealing non-parentality. Indeed, in the case of patient D, if the absence of a pathological GAA expansion had been found in the father, it might initially have been attributed to non-paternity although this could quickly have been disproved by further testing. Thus, testing for a an exonic deletion is warranted if testing of the parent of a patient with genetically proven FRDA, fails to reveal carrier status, particularly if the patient appears to have two equal-sized GAA expansions on long-range PCR. However, the conventional MLPA technique may not pick up all large *FXN* gene deletions and consideration should be given to undertaking alternative techniques to assess the exact breakpoints of a deletion. The currently commercially available MLPA probes may require revision, particularly for the larger exons 1 and 5b to provide more comprehensive coverage.

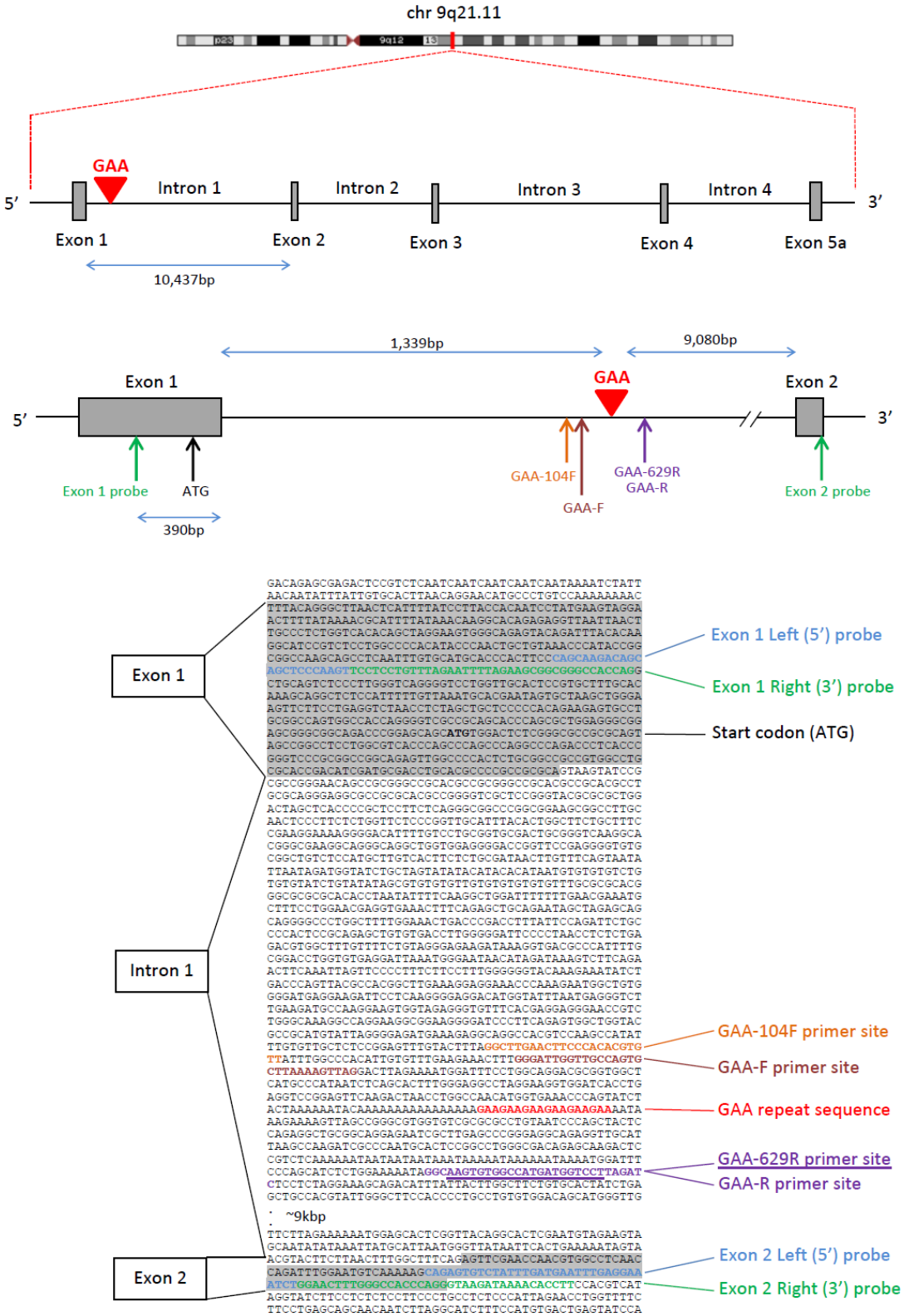


Figure 59: Locations of primer and MLPA probe binding sites for *FXN* exons 1-2

Exons are shaded in grey. Exon 1 & 2 probes relate to MHC-Holland SALSA MLPA P316-B2 Recessive Ataxias probemix. Primers GAA-104F & GAA-629R relate to Filla, *et al.* 1996. Primers GAA-F & GAA-R relate to Campuzano, *et al.* 1996. Note, the GAA-629R and GAA-R binding sites overlap. Genetic data from www.genome.ucsc.edu. *Homo sapiens FXN* transcript variant 1. Chr 9:69,035,259-69,074,213. RefSeq NM_000144.4 Genome GRCh38/hg38.

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Chapter 4 : Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)

4.1 Introduction

The core features of ARSACS are a slowly progressive cerebellar ataxia, spasticity and an axonal-demyelinating peripheral neuropathy, causing incoordination, dysarthria, cerebellar eye signs, limb weakness, muscle cramps, distal amyotrophy, sensory loss, pyramidal signs and skeletal foot abnormalities. Less commonly, hearing and visual loss, dysphagia, urinary symptoms, epilepsy and cognitive deficits have been reported. The condition was first described in, and considered to be confined at relatively high frequency to the descendants of founder populations of, the Charlevoix and Saguenay-Lac Saint Jean regions of North-eastern Québec (Bouchard *et al.* 1978). However, the discovery of the causative *SACS* gene has permitted its identification throughout the world and has extended the diversity of mutations known, and the spectrum of clinical features described (Gomez 2004). ARSACS is now recognized as one of the important causes of autosomal recessive ataxia.

4.2 History

Québec was one of the first regions of North America to be colonized by Europeans and the majority of French Canadians living in Québec Province today are thought to descend from these original founders. As a result, a number of rare neurogenetic disorders show increased prevalence or local variants in this region, including Friedreich's ataxia (FRDA), and other hereditary ataxias, spastic parapareses and neuropathies (Dupré *et al.* 2006; Bouchard *et al.* 2007). Québec City was founded in 1608 under the rule of the French crown and between 1665 and 1725, around forty families migrated from there to the isolated mountainous region of Charlevoix on the north shore of the Saint Lawrence River, with little population exchange with the original colony. Between 1838 and 1855, further families moved from Charlevoix to the more distant Saguenay and Lac Saint Jean regions to the north which had until then been protected for the fur trade (Bouchard *et al.* 1978; see Figure 60).

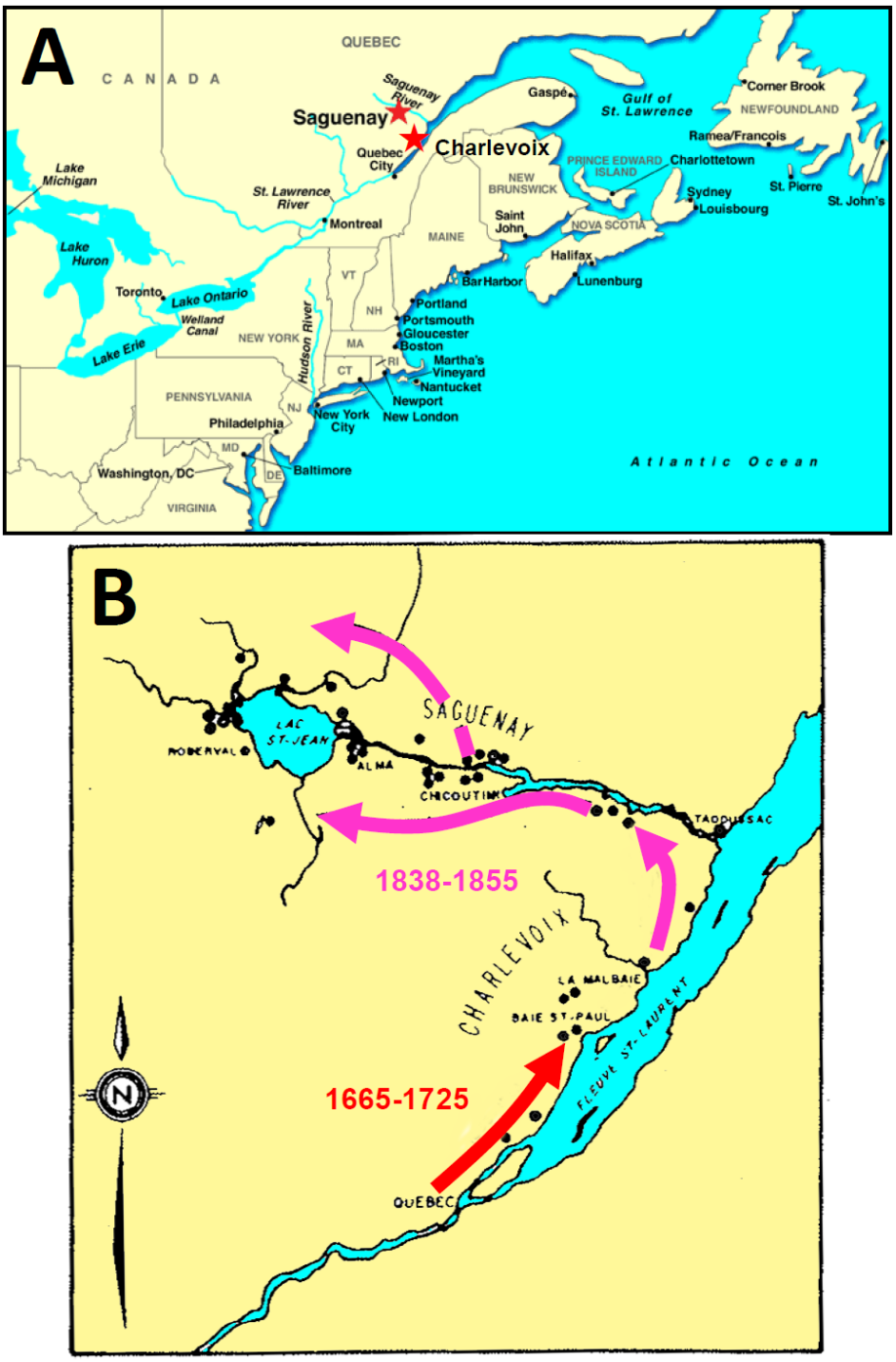

Figure 60: Maps showing (A) location of Charlevoix and Saguenay regions of Québec province, Canada and (B) migration of French Canadian populations
(adapted from Bouchard *et al.* 1978)

The clinical syndrome of ARSACS was first described in 1978 in these populations (Bouchard *et al.* 1978), and this community of more than 300 affected individuals remains the most numerous and most extensively studied to this date. It is estimated that the carrier frequency of *SACS* mutations is 1/22 in Charlevoix and Saguenay-Lac

Saint Jean. The clinical phenotype of the original cases was remarkably homogeneous, probably because more than 92% of carrier chromosomes in these regions have the same mutation (c.8844delT). Although it was initially considered that the condition may have originated in a founder couple living in Québec City around 1650 (Bouchard *et al.* 1978), subsequent analysis has found at least 13 other pathogenic mutations in this community (Thiffault *et al.* 2013).

The causative gene was first described in 2000 (Engert *et al.* 2000) enabling the subsequent identification of cases in Europe, North Africa, Turkey, Japan, Brazil, Israel and other provinces of Canada (Bouhlal *et al.* 2011; Pedroso *et al.* 2011; Blumkin *et al.* 2015; Guernsey *et al.* 2010) with considerable phenotypic heterogeneity, so that now none of the canonical signs of spasticity, ataxia, or origin in Charlevoix-Saguenay is an obligate feature of the condition (Synofzik *et al.* 2013). Alternative names such as 'sacsin-related autosomal recessive ataxia' or 'sacsinopathy' (Takiyama 2007; El Euch-Fayache *et al.* 2003) have therefore been proposed although not widely accepted. However, because of the historical and numerical primacy of the Québecois cases, the world literature is dominated by these initial descriptions and few other large case series have been undertaken with none in the UK. It is therefore vital to characterize this condition clinically and genetically in a large series from the UK which can inform clinical practice and genetic testing in this country and more widely.

4.3 Genetics and Sacsin Protein Function

The causative gene on chromosome 13q12.12 is named *SACS* and was originally thought to contain a single giant exon (Engert *et al.* 2000; see Figure 61). A further 8 exons and a tenth non-coding exon have subsequently been identified upstream of this, forming a 13,737bp open reading frame (Ouyang *et al.* 2006). Nearly 200 different pathogenic mutations have now been described (Thiffault *et al.* 2013; Baets *et al.* 2010; Brais & Dicaire 2015), largely missense, nonsense, frameshift and splice-site mutations spread over exons 1, 2, 4, 6, 7, 8 and 9, but still primarily in the giant exon 10 (Thiffault *et al.* 2013; Brais & Dicaire 2015). Large deletions have also been described causing atypical features such as late onset or prominent hearing loss. These have included an intragenic deletion of exons 3-5 (Baets *et al.* 2010), deletions of the

whole gene (Breckpot *et al.* 2008; Pilliod *et al.* 2015) and deletion of *SACS* and the contiguous genes (Piluso *et al.* 2011; Terracciano *et al.* 2009; Pyle *et al.* 2013) including *SGCG* causing concomitant limb girdle muscular dystrophy type 2c (McMillan *et al.* 2009).

The gene encodes a massive 4,579 amino acid 520kDa protein called sacsin (see Figure 61) which is most highly expressed in cerebellar Purkinje cells; thalamic, midbrain, precerebellar and brainstem nuclei; and large pyramidal forebrain neurones. At a subcellular level, it has been shown to localize to the cystoplasm with a mitochondrial component in SH-SY5Y neuroblastoma cells (Parfitt *et al*. 2009) and to colocalize with mitochondria in the soma, dendrites and axons of cultured hippocampal neurons, COS-7 and HeLa cells (Girard *et al.* 2012).

The true function of the protein is unknown but it has been shown to contain :

- a ubiquitin-like (UBL) domain that can interact with the proteasome;
- three sacsin repeat regions (SSRs) which possess ATPase activity;
- a xeroderma pigmentosum complementation group C binding (XPCB) domain which has been implicated in ubiquitin ligase Ube3A interactions;
- a J-domain (DnaJ motif) associated with chaperone activity and the regulation of the Hsp70 heat shock system; and
- a higher eukaryote and prokaryote nucleotide binding (HEPN) domain that mediates dimerization and nucleotide binding (Girard *et al.* 2012; Kozlov *et al.* 2011; Prodi *et al.* 2013; Romano *et al.* 2013).

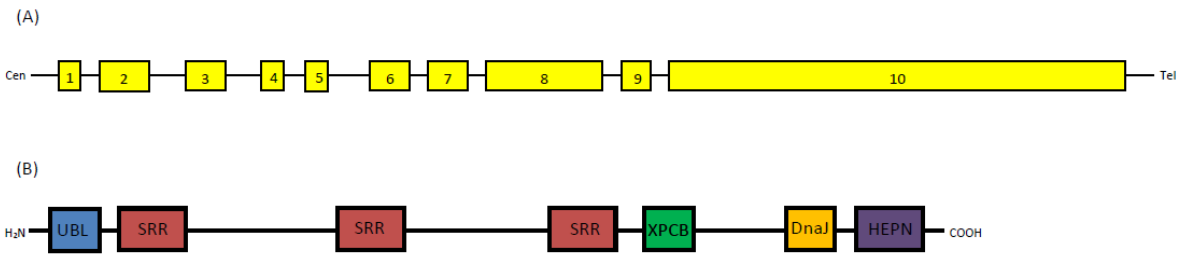

Figure 61: (A) Primary structure of *SACS* gene showing the 10 exons. (B) Domain organization of sacsin protein.

UBL, ubiquitin-like domain; SSR, sacsin repeat region; XPCB, xeroderma pigmentosum complementation group C binding domain; DnaJ, J-domain; HEPN, higher eukaryote and prokaryote nucleotide binding domain

Because of the presence of the ubiquitin-like domain and DnaJ motif, it was postulated that sacsin might participate in the cellular ubiquitin-proteosome and heat shock protein chaperone systems which are crucial in preventing the aggregation of mutant proteins which have been implicated in the aetiology of several neurodegenerative diseases including polyglutamine disorders (Ciechanover & Brundin 2003 ; Dantuma *et al.* 2014) A siRNA-mediated sacsin knockdown model enhanced the toxicity of mutant polyglutamine-expanded ataxin-1 supporting this idea (Parfitt *et al.* 2009).

More recently, cultured immortalized fibroblasts from patients homozygous for the Québecois c.8844delT *SACS* mutation showed balloon-like or bulbed mitochondria suggestive of a mitochondrial hyperfusion seen with enhanced mitochondrial fusion or impaired fission. Coimmunoprecipitation showed an interaction between the N-terminal portion of sacsin and Drp1, a protein involved in mitochondrial fission, suggesting that sacsin might be involved in this process. A siRNA-mediated sacsin knockdown model showed a more interconnected mitochondrial network. In the same model, physiological studies of the mitochondrial membrane potential ($\Delta\Psi_m$) which is generated by oxidative phosphorylation and thus an indicator of mitochondrial function, showed decreased fluorescence with the $\Delta\Psi_m$-sensitive dye tetramethylrhodamine (TMRM), indicating impaired mitochondrial function. Furthermore, recovery of fluorescence by the $\Delta\Psi_m$-sensitive marker MitoTracker after treatment with carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) which disrupts oxidative metabolism, was significantly slower in the sacsin knockdown cells. Cerebellar slice cultures from *SACS*$^{-/-}$ knockout mice also showed impaired $\Delta\Psi_m$ using the immunofluorescent dye JC-1 when compared with controls. Cultured hippocampal neurons from the siRNA knockdown model showed mitochondria clustered in the soma and proximal dendrites compared to a more widespread distribution in control cells, as well as fewer, thicker dendrites. Thus, sacsin may be involved in mitochondrial fission and transport, preservation of mitochondrial membrane potential and dendritic morphology (Girard *et al.* 2012).

Further work on the *SACS*$^{-/-}$ knockout mouse model has shown abnormally increased staining for heavy-chain neurofilament (NFH) in the cell bodies and dendrites of thalamic neurons, cerebellar Purkinje cells and neurons of the deep cerebellar nuclei

and superior olive. This appears to be specific to the non-phosphorylated form of NFH. These changes have also been seen in samples of brain tissue from human ARSACS patients. Studies of spinal motor neuron axons from the *SACS*$^{-/-}$ knockout mice showed decreased mitochondrial motility and markedly increased mitochondrial length. The NFH accumulation and decreased mitochondrial motility were noted before the mitochondrial elongation. *SACS*$^{-/-}$ knockout mice show abnormal gait with progressive motor, cerebellar and peripheral nerve dysfunction, accompanied by early onset progressive loss of cerebellar Purkinje cells followed by spinal motor neuron loss and peripheral neuropathy, indicating that the model is an accurate reproduction of the human disease. These findings further support the notion that sacsin is intimately involved with mitochondrial function and may play a role in the neuronal cytoskeletal network (Larivière *et al.* 2015).

Studies of primary culture of skin fibroblasts from European patients with ARSACS has recently shown altered mitochondrial morphology with an increase in bulbed mitochondria; decreased global mitochondrial mass measured by MitoTracker; decreased mitochondrial DNA (mtDNA) measured by quantitative PCR; and impaired $\Delta\Psi_m$ measured by TMRM. This team felt that mitochondrial network anomalies were so striking that they should be used as a biomarker in determining the pathogenicity of newly proposed *SACS* gene mutations (Pilliod *et al.* 2015). Thus, there is increasing evidence that sacsin plays an important role in mitochondrial function and this may be a key feature of the underlying neurodegenerative process in common with several other neurodegenerative diseases (Burté *et al.* 2014).

4.4 Histopathology

Nerve and muscle biopsies are commonly performed in suspected cases of ARSACS during the often long diagnostic process, but neither provides pathognomonic information. Nerve biopsies most consistently show a marked decrement in large myelinated fibres. More variably, axonal degeneration with condensation of the axoplasm, increased collagen pockets and accumulation of mitochondria and vesicular bodies is seen, sometimes with regenerative axonal sprouting. Thinning of the myelin sheaths with rare onion bulbs may also be observed (Peyronnard *et al.* 1979; El Euch-

Fayache *et al.* 2003; Takiyama 2006). Taken together, these findings suggest an axonal neuropathy associated with some demyelinating features. Muscle biopsies show variation in fibre size with mildly atrophic and hypertrophic fibres, moderate type grouping and variable loss of type I or type II fibres. There are minimal signs of chronic or active denervation (Peyronnard *et al.* 1979; Takiyama 2006; Bouchard 1991). Overall these findings are typical of neurogenic atrophy.

The results of only two post-mortem examinations of patients with ARSACS have been published (Bouchard *et al.* 1998). The first in a young patient who died in a road traffic accident, showed a grossly atrophied superior cerebellar vermis especially in the anterior structures (central lobule and culmen). No changes were seen in the dentate nucleus and inferior olives. The molecular and granular cell layers were thinned with practically absent Purkinje cells. The pyramids, lateral and anterior corticospinal tracts and posterior spinocerebellar tracts all showed significant loss of myelin staining, particularly the lateral corticospinal tracts. The corticospinal changes were more marked in the upper cord, whereas the spinocerebellar changes were more marked caudally. The second, in an older patient, showed similar findings although to a more pronounced degree. Swollen thalamic and cerebellar cortical neurones were seen, suggestive of a storage disorder. Most of these cells showed dense, lipofuscin-like granules within lysosomes, although testing of an extensive panel of lysosomal enzymes was normal. Interestingly, lipofuscin deposits have also been seen in the skin biopsy of a patient with ARSACS performed to exclude Lafora body disease (Stevens *et al.* 2013). Peripheral nerve and muscle biopsies have not shown lipofuscin deposits. The significance of this finding therefore remains unclear.

4.5 Clinical Features

Much of the clinical knowledge of ARSACS is based on the relatively homogeneous Québecois cases. However, subsequently identified cases from elsewhere have demonstrated a genetic and clinical variability which continues to extend the phenotypic description of this condition. The original Québecois cases were published as a series of largely descriptive papers (Bouchard *et al.* 1978; Bouchard 1991) and only

a handful of systematic case series encompassing more than five patients have been published. These are summarized in Table 34.

In the Québecois cases, unsteadiness was noted from commencement of walking (12-18 months old) which was rarely delayed (Bouchard *et al.* 1998; Bouchard 1991). 80% initially presented because of walking difficulties and a tendency to fall. At first presentation, approximately 60% were found to have limb ataxia, 80% showed some pyramidal involvement and 50% had both pyramidal and cerebellar involvement. There was no clinical evidence of neuropathy at presentation in the form of pes cavus or intrinsic hand muscle wasting (Duquette *et al.* 2013). There is some evidence that age at onset may be a little later in non-Québecois cases, particularly in Japanese and Tunisian cases (Takiyama 2007). In a series of 17 Belgian patients, 29% had onset at or after age 20 with one as late as 40 (Baets *et al.* 2010). There is no male-female preponderance.

Thus, limb and gait ataxia are early signs followed by spasticity, which is more prominent in the lower limbs. Spasticity and ataxia affect speech, which is often slightly slurred in childhood and can become explosive in adulthood. Dysphagia is usually mild or absent (Bouchard 1991; Prodi *et al.* 2013). Plantar reactions are frequently upgoing from childhood. Eye movements show horizontal bidirectional nystagmus, saccadic alterations of smooth ocular pursuit and saccadic dysmetria (Bouchard 1991). Supranuclear gaze palsy has been described in one case (Stevens *et al.* 2013). In the Québecois cases, by the age of 10 more than 90% showed both pyramidal and cerebellar involvement (Duquette *et al.* 2013). Muscle cramps may be a troublesome feature. Progression of symptoms is slow. In the Québecois cases, only 4% used a wheelchair before the age of 18 (Duquette *et al.* 2013). Mean age to being wheelchair-bound was around 40 (range 17-58) and to death around 50 (range 21-72) (Bouchard 1991).

Table 34: Frequencies of clinical features in published case series

	Bouchard *et al.* 1978	El Euch-Fayache *et al.* 2003	Takiyama 2006	Vermeer *et al.* 2008	Baets *et al.* 2010	Prodi *et al.* 2012	Synofzik *et al.* 2013	Pilliod *et al.* 2015
Origin	Canada	Tunisia	Japan	Netherlands, Turkey, UK	Belgium, Morocco, Serbia, Hungary	Italy	Germany, Turkey, Greece, Macedonia	France, Turkey, Maghreb, Portugal, Italy, Australia, Poland
Genetic diagnosis	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
No. of patients	42	18	8	23	17	14	13	47
No. of families	24	4	5	16	11	13	9	39
Male:female	1 : 0.7	1 : 0.4	1 : 1	1 : 0.9	-	1 : 1.3	1 : 1.2	1 : 1.6
Age at onset (years)[a]	No patients ever walked normally	4.5±3.3 (1-14)	5.5±1.6 (3-8)	3.5±3.1 (1-12)	13.1±11.1 (1-40)	3.6±2.6 (1-32)	7.6±8.7 (1-28)	5.1±7.4 (0-30)
Age at examination (years)[a]	30.5±11.2 (9-52)	32.6±11.5 (16-55)	35.3±6.1 (26-44)	40.3±10.9 (26-58)	34.7±11.1 (12-52)	32.2±9.4 (18-46)	28.7±13.5 (6-50)	29.7±12.9 (8-53)
Disease duration (years)[a]	Lifelong	28.1±9.8 (11-45)	29.5±6.3 (22-39)	36.2±9.9 (25-56)	22.0±6.6 (11-35)	26.6±10.3 (12-44)	23.0±9.4 (4-39)	24.9±12.1 (7-52)
Age at wheelchair use (years)[a]	-	30.0±0.8 (29-31)	36.8 (30-43)	-	-	-	-	-
Wheelchair-bound (%)	-	22	-	68	65	-	-	22
Typical initial symptoms	Never walked properly	Cerebellar syndrome involving gait & movement	Gait disturbance & falls	Slowly progressive cerebellar ataxia	Multiple symptoms	Gait instability & falls	Gait disturbance	Unsteadiness
Progression of symptoms	Progressive with periods of stability	Spasticity later	Progresses in late teens/early 20s	Subsequent LL spasticity, then neuropathy	-	Spasticity developed later	-	-
Mobility	-	22% wheelchair use	-	32% aids; 68% wheelchair use	18% unaided; 18% aids; 65% wheelchair use	64% autonomous; 36% with aids	-	46% unaided; 32% aids; 22% wheelchair use

Spastic gait (%)	-	-	-	100	-	79	-	-
UL spasticity (%)	-	-	-	24	-	-	-	12
LL spasticity (%)	-	-	(75)[c]	100	59	100	(67)[c]	65
Dystonia (%)	-	-	-	13	-	-	-	4
Ataxic gait (%)	100	-	-	-	-	79	-	-
UL ataxia (%)	-	-	-	100	-	-	83	-
LL ataxia (%)	-	-	(100)[c]	100	(88)[c]	-	75	(100)[c]
Dysarthria (%)	100	78	100	-	59	-	50	-
Dysphagia (%)	36	-	-	30	-	7	-	6
Nystagmus (%)	100	100	100	-	65	-	67	83
UL reflexes (%)[b]	-	-	(H:75)[c]	N:59 A:35 H: 6	(H:59)[c]	H:36	-	N:28 A:35 H:37
Patellar reflexes (%)[b]	-	N:0 A:0 H:100	-	N:32 A:26 H:42	-	-	-	N:9 A:23 H:68
Ankle reflexes (%)[b]	N:7	N:0 A:100 H:0	-	N:0 A:95 H:5	-	N:0 A:57 H:43	-	N:9 A:61 H:30
Extensor plantar responses (%)	100	-	100	-	82	100	58	78
Sensory loss (%)	100	100	-	-	53	-	-	68
UL weakness (%)	-	-	-	-	41	-	-	33
LL weakness (%)	-	-	-	-	82	-	-	67
UL amyotrophy (%)	21	67	(88)[c]	-	-	100	-	19
LL amyotrophy (%)	-	67	-	-	-	100	-	35
Scoliosis (%)	0	11	-	-	-	36	-	9
Foot abnormalities (%)[d]	86	44	75	-	35	-	-	57
Hand abnormalities (%)[e]	'sometimes seen'	-	63	-	-	-	-	-
Mitral valve prolapse (%)	57	-	-	0	6	-	-	-
Other cardiac involvement (%)	0	0	-	8[f]	-	-	-	-
Urinary problems (%)	64	>65%	-	48	-	21	77	23
Hearing loss (%)	-	-	-	-	-	14	0	13
Cognitive impairment (%)	See note[g]	-	75	7[h]	12	14	-	9
Epilepsy (%)	5[i]	-	-	4	6	0[j]	-	9

Rating scales[k,l]	-	ICARS: 33.6±15.8 (4-60)	-	SARA: 22.2±5.1 (14-31.5)	-	-	SARA: 12.4±8.7 (1-25)	-
EMG/NCS		100% showed severe to moderate axonal neuropathy with demyelinating features	100% showed moderate to severe sensory motor axonal neuropathy with demyelinating features	100% sensory axonal neuropathy; 54% secondary demyelinating features	91% abnormal with predominantly mixed axonal-demyelinating	100% showed severe sensory motor axonal neuropathy with demyelinating features	89% abnormal with marked axonal-demyelinating sensory motor neuropathy	56% demyelinating; 25% axonal; 11% mixed; 8% other polyneuropathy
<u>Imaging</u> **Cerebellar atrophy (%)**	-	-	100	65	47	100	89	90
Pontine hypodensities (%)	-	-	-[m]	-	-	100	100	38
Spinal cord atrophy (%)	-	-	38	-	6	89	NR	20
<u>Fundoscopy</u> **Retinal hypertrophy (%)**	100	11	63	100	13	0	17	19

[a]Expressed as mean±SD (range)
[b]N=normal, A=absent or hyporeflexic, H=hyperreflexic
[c]Site not specified (UL vs LL)
[d]Pes cavus, pes planus, claw toes, hammer toes, varus or equinus deformities
[e]Claw hands, swan-neck deformities
[f]Right bundle branch block on ECG in 1 case
[g]21 patients underwent psychometric evaluation showing deficits in non-verbal scales, particularly scales of object assembly & digit symbols
[h]One patient had meningitis with epilepsy & another strokes which may independently explain cognitive decline
[i]Subsequently described in Bouchard *et al.* 1991 in which 5 out of 104 patients had epilepsy
[j]Five patients had non-specific EEG abnormalities 'mainly epileptiform activity'
[k]ICARS=International cerebellar ataxia rating scale (0-100 with higher number representing greater involvement)
[l]SARA=Scale for the assessment and rating of ataxia (0-40 with higher number representing greater involvement)
[m]In a subsequent overlapping series, 100% of patients showed pontine hypodensities (Shimizaki *et al*. 2013)
- Not known or not recorded
UL=upper limb ; LL=lower limb
EMG=Electromyogram ; NCS=Nerve conduction studies

From childhood, deep tendon reflexes are frequently increased, but by adulthood, may diminish or become absent due to progressive neuropathy. Ankle jerks are commonly absent whilst knee jerks may be hyperreflexic but patients may have a very mixed and asymmetric picture. Sensory deficits usually appear later and progressively into adulthood, involving vibrational sense more than proprioception and cutaneous sensation. Distal amyotrophy also appears progressively later in the condition (Bouchard 1991). The combination of early spasticity and progressive neuropathy commonly causes skeletal abnormalities of the foot including pes cavus, talipes equinus or varus, and hammer or clawed toes. Unlike FRDA, spinal scoliosis is not a prominent feature (Bouchard 1991) but has been described in Tunisian (El Euch-Fayache *et al.* 2003) and Italian (Prodi *et al.* 2013) series. Straight dorsal spine has been described in a Spanish series (Gazulla *et al.* 2012). In the hands, swan-neck deformity of the fingers and claw hands has been described (Bouchard *et al.* 1978; Takiyama 2006) with dystonia sometimes causing abnormal posturing of the hands and neck (Vermeer *et al.* 2008).

Cognition is generally preserved particularly on tests of verbal function, but visuospatial handling may be diminished and deteriorate with time (Bouchard *et al.* 1998). Cognitive impairment may be a more prominent feature amongst non-Québecois patients, with intellectual impairment and dementia described in patients from Japan, Italy and Turkey (Takiyama 2006). Although cerebellar eye signs may cause visual disruption, optic nerve and retinal function are not generally affected with normal acuity, fields and colour vision, despite the presence of thickened retinal nerve fibres (see Chapter 8) (Bouchard 1991). Hearing loss is not generally found but may be more prominent amongst cases involving *SACS* gene deletions (Breckpot *et al.* 2008; Prodi *et al.* 2013; Terracciano *et al.* 2009).

Bladder and bowel symptoms are not well-studied in ARSACS although urinary urge incontinence has been most commonly described (Bouchard *et al.* 1978; Synofzik *et al.* 2013; Bouchard 1991; Prodi *et al.* 2013; Vermeer *et al.* 2008). Faecal incontinence and constipation may also be a problem in patients with long disease duration (Bouchard 1991). Co-existent epilepsy has been described in a minority of cases and it remains unclear whether this is a definite association (Stevens *et al.*

2013; Vermeer *et al.* 2008). It appears more common in the Québecois cases, occurring in more than 15% in one series (Duquette *et al.* 2013). Frequent abnormal electroencephalographic features have also been described (see 4.7 below).

Currently no clinical diagnostic criteria exist for ARSACS. The descriptive clinical features published by Bouchard, *et al* (1991, 1998) have come closest to this, although may be more representative of the Québecois cases (see Table 35).

Table 35: Clinical Features of ARSACS (modified from Bouchard *et al.* 1991 & 1998*)*

Onset Unsteadiness at gait initiation
Progressive Signs Mostly spastic ataxia of the four limbs Slurred and dysrythmic speech Discrete to severe distal amyotrophy Absent ankle jerks after 25 years of age
Early Non-Progressive Signs Increased deep tendon reflexes Bilateral abnormal plantar response Marked saccadic alteration of ocular pursuit At funduscopy: prominent retinal nerve fibres radiating from the disc and embedding retinal vessels
Positive Diagnostic Tests CT or MRI: atrophy of the superior vermis; progressive atrophy of the cerebellar hemispheres and of the cervical spinal cord NCS: axonal neuropathy with absent sensory action potentials and low motor conduction velocities

4.6 Imaging

The predominant radiological manifestations of ARSACS on MRI and CT are marked atrophy of the superior cerebellar vermis with consequent enlargement of the supravermian cisterns and cisterna magna (see Figure 62) (Baets *et al.* 2010; Takiyama 2006; Prodi *et al.* 2013; Gazulla *et al.* 2012). Posterior fossa arachnoid cysts are also sometimes reported (Synofzik *et al.* 2013). While such prominent cerebellar atrophy is uncommon in FRDA, these findings are also seen in other causes of spinocerebellar ataxia (SCA). More specific appear to be the paramedian, bilaterally symmetrical, parallel, linear hypointensities in the pons on T2 and T2-

FLAIR MRI sequences (Gazulla *et al.* 2012; Martin *et al.* 2007) which some have called 'pontine tigroid hypointensities' (Terracciano *et al.* 2010). Associated with these may be bilateral T2-FLAIR *hyper*intensities of the lateral pons at the level of the middle cerebellar peduncles (MCPs) (Synofzik *et al.* 2013). The hypo- and hyper-intensities may extend into the MCPs (Shimazaki *et al.* 2013). The pons generally may be bulky (Prodi *et al.* 2013) and the MCPs thickened (Synofzik *et al.* 2013; Prodi *et al.* 2013; Gazulla *et al.* 2012). The pontine striations have not been reported in other causes of ataxia or spastic paraparesis, making them useful in distinguishing ARSACS from these conditions when present. Diffusion tensor imaging (DTI) has shed some light on the underlying nature of these changes and the cause of symptoms in ARSACS, with hyperplastic pontocerebellar fibres at the same level as thin and abnormally placed pyramidal tracts, suggesting that the former may be compressing the latter (Prodi *et al.* 2013; Gazulla *et al.* 2012).

Atrophy of the superior cerebellar peduncles (SCPs), medulla, cervical and thoracic cords has also been observed (Bouchard 1991; Prodi *et al.* 2013), although again not consistently, particularly in non-Québecois cases (Shimazaki *et al.* 2007). More widespread cerebral atrophy, particularly bilaterally in the parietal lobes (Synofzik *et al.* 2013), may be seen later in the course of the condition but is not as prominent as the cerebellar or cervical atrophy (Baets *et al.* 2010; Bouchard *et al.* 1998). Thinning of the corpus callosum and a rim of T2 hyperintensity around the thalami have also variably been reported (Synofzik *et al.* 2013; Prodi *et al.* 2013). No white matter abnormalities have been seen in either brain or spine (Takiyama 2006; Bouchard 1991) except in one atypical case in which the explanation was felt to be concomitant multiple sclerosis (Terracciano *et al.* 2010). Single photon emission computed tomography (SPECT) has shown decreased blood flow in the superior cerebellar vermis (Shimazaki *et al.* 2007).

Thus, the salient imaging features of ARSACS are prominent early superior vermian cerebellar atrophy, thinning of the predominantly cervical spinal cord and pontine linear hypointensities.

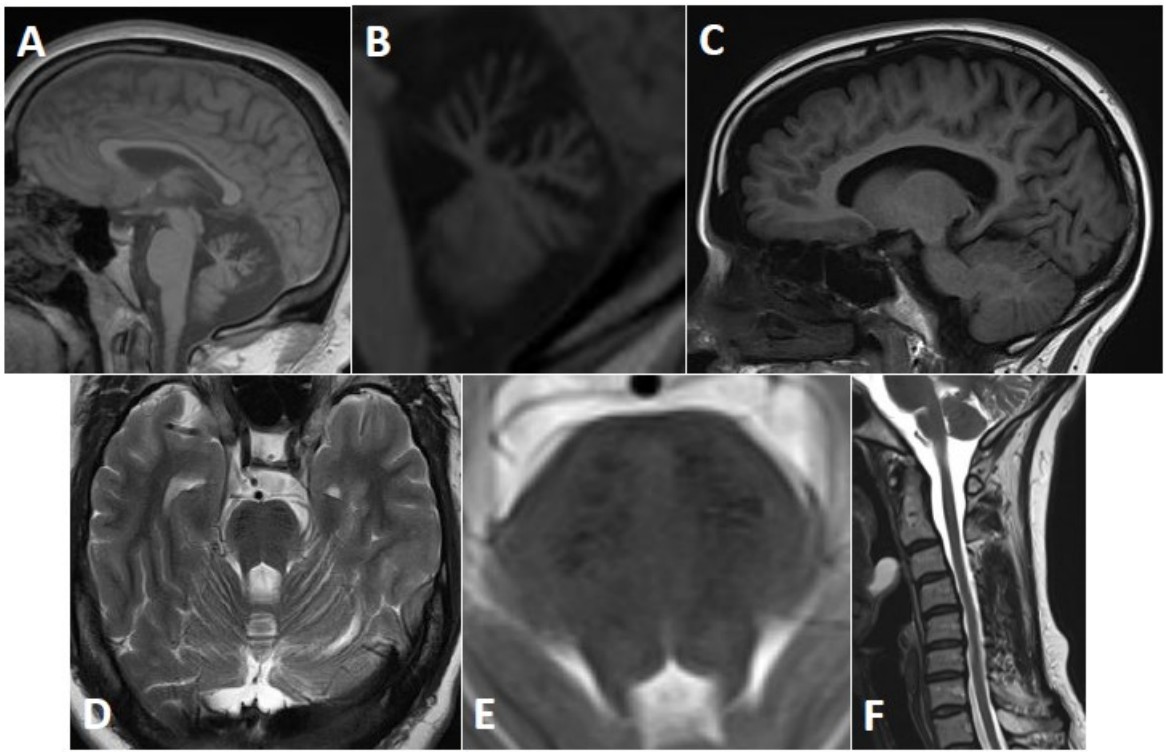

Figure 62: Radiological features of ARSACS

A) Sagittal T1 MRI showing superior vermian atrophy and corpus callosal thinning ; B) Sagittal T1 MRI showing superior vermian atrophy ; C) Sagittal T1 MRI showing generalized cerebral volume loss, most preponderant in the parietal lobe ; D) Axial T1 MRI showing pontine hypointensities ; E) Axial T2 MRI showing pontine hypointensities ; F) Sagittal T2 MRI showing thinning of the cervical spinal cord

4.7 Neurophysiological studies

Nerve conduction studies show increased distal motor latency and decreased conduction velocities which are more pronounced in the lower limbs than the upper limbs. Typical median nerve conduction velocities are 29-44ms^{-1} and peroneal nerve 17-35ms^{-1}. This appears to distinguish ARSACS from FRDA in which motor conduction velocities are usually preserved. Motor conduction slowing appears early in life with progressive degeneration which may make compound motor action potentials impossible to detect at the feet by middle age. Sensory nerve conduction is usually of low amplitude or unrecordable, especially in the lower limbs (Peyronnard *et al.* 1979; El Euch-Fayache *et al.* 2003; Bouchard 1991; Gazulla *et al.* 2012). Electromyography shows fibrillations, occasional fasciculations, increased polyphasic action potentials and decreased or absent recruitment,

indicating chronic denervation of distal muscles early in the disease process (Bouchard 1991; Gazulla *et al.* 2012). Sympathetic skin responses are normal. Somatosensory evoked potentials show a dispersed and delayed cortical response indicating slowed central sensory conduction. Brainstem and visual evoked potentials show increased latencies even in the absence of auditory or visual symptoms (Bouchard 1991; Gazulla *et al.* 2012). Electroretinography is normal (Prodi *et al.* 2013; Desserre *et al.* 2011). Transcranial magnetic stimulation also shows marked delay in the central pathways (Bouchard 1991). Thus, neurophysiological studies suggest an early demyelinating sensorimotor neuropathy with progressive axonal degeneration, and involvement of the central sensory and motor pathways.

Electronystagmography (ENG) most commonly shows horizontal gaze-evoked nystagmus and impairment of smooth ocular pursuit. There is also impairment of optokinetic nystagmus and defective fixation suppression of caloric nystagmus. Saccades are dysmetric but saccadic velocities are normal (Bouchard 1991).

Electroencephalography (EEG) reveals abnormalities in 40-60% of patients although frank epilepsy is much less commonly reported (Bouchard 1991; Prodi *et al.* 2013). These abnormalities may be non-specific findings indicating involvement of cortical and subcortical structures similar to those reported in FRDA.

4.8 Differential Diagnosis

FRDA is the commonest cause of autosomal recessive cerebellar ataxia and the chief condition in the differential diagnosis of ARSACS. Retained or brisk reflexes and spasticity are rarely features of FRDA except in atypical late-onset cases known as Friedreich's ataxia with retained reflexes (FARR) (Parkinson *et al.* 2013). Cerebellar atrophy is more pronounced in ARSACS. A striking feature which distinguishes ARSACS from FRDA and other mitochondrial disorders, is the absence of extraneurological features such as diabetes, cardiomyopathy and scoliosis. The electrocardiogram in ARSACS is typically normal, as compared to the frequent existence of repolarization abnormalities in FRDA. Although mitral valve prolapse

was described in the original cases of ARSACS (Bouchard *et al.* 1978), this finding has not been replicated in subsequent studies of families outside Québec.

Ataxia with oculomotor apraxia (AOA) may be distinguished from ARSACS because of the presence of oculomotor apraxia, dystonia, chorea and the absence of pyramidal features. AOA type 1 is associated with low levels of serum albumin and elevated levels of low density lipoproteins (LDLs), whilst AOA type 2 shows elevated levels of α-fetoprotein (AFP). Ataxia telangiectasia has many features in common with AOA together with cutaneous and scleral telangiectasiae, diabetes, immunodeficiency and sensitivity to radiation causing tumours (Desserre *et al.* 2011).

Late-onset Alexander's disease may have onset in adolescence and have a presentation similar to ARSACS. Cerebellar atrophy is less prominent and there may be periventricular white matter changes on MRI which are not seen in ARSACS. Cerebrotendinous xanthomatosis has onset in infancy but is often associated with diarrhoea, cataracts and tendon xanthomata, and is identifiable because of elevated serum cholestanol and urinary bile alcohols. Of the hereditary spastic parapareses (HSPs), HSP7 may be one of the most common to be complicated by ataxia, although onset is generally in adulthood. HSP 11, 15, 20, 21 and 27 may also present with ataxia, although often show distinguishing features (de Bot *et al* 2012; Salinas *et al.* 2008). In the spinocerebellar ataxias (SCAs), cerebellar ataxia generally predominates and inheritance is autosomal dominant. Amongst these, SCA1 and SCA3 (Machado-Joseph disease) are the most common which can present with a spastic paraparesis, however in both of these conditions, age at onset is in adulthood.

If neuropathy dominates the clinical picture over spasticity and ataxia, Charcot-Marie-Tooth (CMT) disease may be considered (Synofzik *et al.* 2013). A number of other rare causes of ataxia or spastic paraparesis may need to be considered including spastic ataxia types 1 to 5 (SPAX1-5), abetalipoproteinaemia, ataxia with vitamin E deficiency (AVED), ataxia with coenzyme Q_{10} deficiency, Niemann-Pick

disease type C, Refsum's disease and autosomal recessive cerebellar ataxia type 2 (ARCA2), for which genetic or metabolic tests are available (Anheim *et al* 2012).

Once acquired causes of spastic ataxia have been excluded, the combination of age at onset, suspected mode of inheritance, associated clinical, neuroimaging, neurophysiological and other features should guide genetic testing. In the future, next generation (Németh *et* al. 2013) and whole exome sequencing will allow parallel testing of multiple suspected genes, although it will remain vital to interpret the results in terms of pre-existing suspicions from careful clinical phenotyping.

The triad of early-onset ataxia, spasticity and axonal-demyelinating neuropathy, together with sporadic or autosomal recessive inheritance, prominent superior cerebellar, cervical atrophy and pontine linear hypointensities on MRI and no extraneurological features, should provoke the suspicion of ARSACS. These clinical features will be explored in a large cohort of patients seen at NHNN in Chapter 5. The presence of thickened retinal nerve fibres on OCT may be a sensitive marker of ARSACS. This feature will be explored in Chapter 6.

4.9 References for Chapter 4

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Chapter 5 : Natural History of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)

5.1 Introduction

Chapters 5 and 6 of this thesis are formed from data as part of a natural history study of ARSACS in the UK analogous to EFACTS, and a study of the sensitivity and specificity of ocular coherence tomography (OCT) in the diagnosis of ARSACS. Chapter 6 also includes a description of an Illumina TruSeq Custom Amplicon panel designed to detect mutations in a range of genes causing spastic ataxia.

5.2 Method

5.2.1 Ethics, Recruitment & Funding

The project was approved by the London Brent Research Ethics Committee (reference 12/LO/1291) and subsequently amended. Patients with ARSACS were identified from the records of the NHNN and contacted initially using a standard letter enclosing the Participant Information Sheet (PIS). Telephone contact was subsequently made if necessary. Advertisements were placed in The Ataxian, the newsletter of Ataxia UK, and in the electronic newsletter of the Association of British Neurologists (ABN). Through the latter method, patients were identified via neurologists in Newcastle and Southampton. One patient was referred directly from a collaborator in Switzerland. Funding for travel expenses was provided by Ataxia UK.

5.2.2 Neurological Assessment

Patients were invited to attend a research clinic at the NHNN. Those who were unable or unwilling to attend were offered a home visit. In one case a patient was seen in the local District General Hospital. Each patient provided signed informed consent after having received the PIS. The clinical assessment lasted approximately 2 hours. Each patient provided basic demographic details including country of birth, ethnic origin, level of educational achievement, occupational history, marital and family status. A

structured history of the disease was elicited including age and nature of first symptoms, date of clinical and genetic diagnosis, and significant disability milestones such as age of first falls, and intermittent or permanent use of aids to mobility or wheelchair. Age at onset was taken as the date at which the patient first experienced symptoms adjudged by the examiner to be consistent with ARSACS. Details of associated symptoms were recorded such as visual impairment, hearing loss, scoliosis and skeletal hand and foot abnormalities. A detailed family history was recorded including the relation, age, age at onset and age at death of family members diagnosed with or suspected of having ARSACS and other causes of ataxia. A detailed structured past medical history was recorded. A detailed history was recorded of present and past use of medications, vitamins, supplements, alcohol, smoking and recreational drugs.

Functional disability was assessed using the 9-field Activities of Daily Living (ADL) section of the Friedreich's Ataxia Rating Scale (FARS) and the Spinocerebellar Degeneration Functional Score (SDFS), as described in Chapter 2. The SDFS has been used extensively the Spastic ataxia research network SPATAX (https://spatax.wordpress.com/the-network-2/) in the assessment of patients with spastic ataxia (Pilliod *et al.* 2015). A detailed structured neurological examination was performed and recorded including assessments of muscle atrophy, tone, power, deep tendon reflexes, pin-prick, joint position and vibrational sensation. Ataxic signs were specifically assessed using the Scale for the Assessment and Rating of Ataxia (SARA) and the Spinocerebellar Ataxia Functional Index (SCAFI), as described in Chapter 2. Where available, a detailed review was made of the patients' paper and electronic medical records. The results of previous neurophysiological and radiological investigations were collated where available, but were not collected prospectively as part of this study.

5.2.3 Genetic Studies

Genomic DNA from peripheral blood lymphocytes was extracted using standard procedures in the Department of Neurogenetics, NHNN. Subsequent *SACS* gene analysis was performed by the diagnostic service in the Department of Human Genetics, Radboud University, Nijmegen Medical Centre, Nijmegen, Netherlands, using

previously described methods (Vermeer *et al.* 2008). In a small number of cases, the diagnosis was made using the Illumina TruSeq Custom Amplicon technique (Illumina, San Diego, CA USA) described more fully in chapter 6. These cases were confirmed in a commercial laboratory (Centogene, Rostock, Germany, http://www.centogene.com/). The gene variants are annotated according to reference sequences NM_014363.4 (cDNA) and NP_055178.3 (protein).

In silico techniques were used to predict the pathogenicity of the novel missense variants identified. These are divided into four principal categories: (1) presence in variant databases; (2) DNA sequence conservation data; (3) prediction of physicochemical properties of amino acid change; and (4) predictions of protein stability. Exact sources (websites, etc) of these techniques are given in the footnotes to Table 39.

The presence of a variant at significant frequency in databases of unaffected individuals provides evidence of non-pathogenicity. Hence, one would expect a proposed pathogenic variant for a rare condition to be absent or appear in extremely low frequency in such databases. This frequency is conventionally recorded as the frequency of the least common allele, called the minor allele frequency (MAF). Three commonly used databases were searched for the presence or absence of the missense variants found in the study. The 1000 Genomes Project used the genomes of 1,092 individuals from 14 populations from Europe, East Asia, Sub-Saharan Africa and the Americas, analyzed through a combination of low-coverage whole-genome sequence data, targeted deep exome sequence data and dense single nucleotide polymorphism (SNP) genotype data, to identify 38 million SNPs, 1.4 million bi-allelic indels and 14,000 large deletions (The-1000-Genomes-Consortium 2012). The National Heart, Lung and Blood Institute Grand Opportunity Exome Sequencing Project (NHLBI GO ESP) sequenced the exomes of 15,336 genes from 6,515 individuals of European American or African American origin, resulting in a dataset of 1.1 million autosomal protein-coding variants (Fu *et al.* 2013). The National Center for Biotechnology Information (NCBI) Database of Single Nucleotide Polymorphisms (dbSNP) is a web-based central public repository of genetic variation data covering a wide variety of organisms submitted by registered individuals and organizations (Sherry *et al.* 1999). The variants

include SNPs, deletion/insertion polymorphisms (DIPs), short tandem repeats (STRs) and multiple nucleotide polymorphisms (MNPs). However, because there is no assumption about the minimum allele frequency, the data are known to contain both pathogenic and non-pathogenic variants (Wallis *et al.* 2013).

Pathogenic variants are often found at sites which show evolutionary conservation in homologous genes across many species as these sites are considered to encode structurally or functionally active regions of the encoded protein. Correspondingly, evolutionary variation will be tolerated at sites which encode less important regions of the protein, and these are less likely to be sites of pathogenic variation. Various algorithms have been developed to compare multiple sequences of homologous genes in alignment (multiple sequence alignments, MSA), and generate a score to predict the likely pathogenicity or tolerance of the variant in question. The scores vary in the number and selection of MSAs used, the extent to which flanking DNA sequences are included, the use of different statistical models of evolution, and the degree to which they invoke the phylogenetic relationships between the species in question. Some use pre-defined annotations to train the model in detecting variation in particular regions of the genome, whilst others act in an undirected manner. A variety of statistical methods are used to generate the final scores (Pollard *et al.* 2010).

Genome Evolutionary Rate Profiling (GERP) measures the degree of evolutionary conservation or constraint by comparing the number of substitutions expected at a particular site under neutral evolution with those observed in reality over 29 mammalian species. This results in a 'rejected substitutions' score (Cooper *et al.* 2005). Positive scores represent fewer substitutions than would be expected and hence evolutionary constraint. Therefore, the greater the positive score, the greater the likelihood of pathogenicity. Negative scores suggest that a site is probably naturally varying under neutral evolution but cannot be used to deduce accelerated evolution for statistical reasons. The maximum genome-wide score of about 5.8 applies to sites that are perfectly conserved across all sequenced mammals (Cooper & Shendure 2011), although values greater than this are possible depending on the statistical parameters employed.

The Phylogenetic P-Values (PhyloP) program similarly compares the variation in single nucleotide sites across different species using MSAs (Siepel *et al.* 2006). The score generated represents the $-\log_{10}$ of the *p*-value from a null hypothesis test of neutral evolution using a statistical technique called the hidden Markov model. Unlike GERP, PhyloP can measure both acceleration (faster evolution than expected under neutral drift) as well as conservation. Positive values represent conservation, and negative, acceleration. As with GERP, the greater the positive score, the greater the likelihood of pathogenicity.

PhyloP measures conservation at the single nucleotide level, ignoring the effects of flanking nucleotides. By contrast the Phylogenetic Analysis with Space/Time Models Conservations (PhastCons) program uses the same methodology based on a two-state phylogenetic hidden Markov model to estimate the probability that each nucleotide belongs to a more widely conserved element (Siepel *et al.* 2005). The resulting score represents a probability between 0 and 1, the closer the value to 1, the more likely the nucleotide is conserved, and hence if the site of a variant, the more likely to be pathogenic.

The third group of techniques used to assess the predicted pathogenicity of a variant involve assessments of the degree of physicochemical change between the amino acids in the wild type and mutant proteins. The Grantham score uses differences in three properties (composition, polarity and molecular volume) between each of 20 amino acids weighted so that the result correlates with the relative frequency of substitution in commonly found proteins (Grantham 1974, McLachlan 1972). Composition is defined as the ratio of the atomic weight of the non-carbon elements of the side chain to that of the carbon atoms in the side chain. The resulting score varies between 5 and 215 with the larger value representing greater change and therefore greater likelihood of pathogenicity. The smallest change is between leucine and iso-leucine, and the greatest between tryptophan and cysteine. Grantham scores are designated as conservative (0-50), moderately conservative (51-100), moderately radical (101- 150) or radical (≥151) according to the classification proposed by Li (Li *et al.* 1984).

Sorting Intolerant From Tolerant (SIFT) is a homology-based program which takes into account both DNA conservation data and chemical differences in the predicted amino acid change (Ng & Henikoff 2001, Ng & Henikoff 2002, Ng & Henikoff 2003). SIFT searches for similar DNA sequences, chooses closely related sequences that may have similar function, and obtains MSAs for these sequences. SIFT considers the extent of conservation and the nature of the amino acid change, so that if all sequences contain the same amino acid at a particular position, the program will consider any variance from this as deleterious, whereas for example, if multiple different hydrophobic amino acids are found, only variances including non-hydrophobic amino acids would be considered deleterious. The program calculates normalized probabilities for all possible substitutions and predicts those less than 0.05 to be damaging, and those greater, to be tolerated.

The final category of pathogenicity predicting software employed uses DNA sequence data as well as predictions about the physical and chemical properties of the final protein produced. The Polymorphisms Phenotyping version 2 (PolyPhen2) program first selects a set of homologous sequences using a clustering algorithm and then constructs and refines the MSA using algorithms which predict features of the tertiary structure of the protein (eg coiled coils, transmembrane helices and low-complexity regions). The program then uses eight sequence-based methods (such as changes in the residue side chain volume and whether the variant happened amongst CpG repeats) and three structure-based methods (change in accessible surface area of amino acid residue, change in hydrophobic propensity and the crystallographic B-factor) to predict the functional significance of an amino acid change (Adzhubei *et al.* 2010). Two datasets were used to train the program. For Mendelian disorders which usually involve a significant change in protein function compared to other forms of human variation, use of the HumVar dataset (Capriotti *et al.* 2006) which treats some mildly deleterious variants as non-damaging, was found to be most appropriate. The program calculates a probability between 0 and 1 that the variant is damaging, and reports the prediction as benign (>20% false positive rate, FPR), possibly damaging (10-20% FPR) or probably damaging (<10% FPR).

Because it is recognized that there are inconsistencies between different pathogenicity prediction tools and no one technique should be relied upon, a multifactorial program, Mutation Taster (Schwarz *et al.* 2014), was also used which integrates many of the above and other methods into a final prediction. The program contains variant data from the 1000 Genomes Project, ClinVar, dbSNP, HGMD Public and HapMap, and tests for regulatory features, splice site variants, evolutionary conservation, and alterations in protein length and other features. A variety of models are used to integrate the above tests according to the nature of the amino acid alteration, with the *'simple_aae'* model used for the missense variants found in this study which is appropriate for single amino acid substitutions. A trained Bayes classifier is used to assign a prediction of pathogenicity using disease-causing mutations known from OMIM, HGMD and the literature. The program reports a prediction of 'polymorphism' (ie harmless) or 'disease causing' together with a probability in the range 0 to 1. If the variant is already known to be harmless or pathogenic, the term 'automatic' is appended. The program also evaluates a number of allied tests (eg Grantham score) not directly used in the prediction, but which can be quoted as further supportive evidence.

5.3 Results

5.3.1 Demographics

Twenty-six patients were assessed between November 2012 and June 2014. Fifteen were seen at NHNN, one at Ipswich Hospital and ten as part of home visits. The basic demographic details of the patients including gender, ethnicity, country of birth, marital status, offspring, education and employment are summarized in Table 36, and the locations of the UK patients in Figure 63. Basic demographic details of each patient individually are given in Table 37. The range of ages at which the patients were examined as part of the study is shown in Figure 64 with ages from 21 to 69 years. It should be noted that the ethics permission of the project did not permit recruitment of patients below the age of 16 and so these necessarily do not appear in this project. The duration of disease at examination is shown in Figure 65 with a range from 8 to 56 years. This is also influenced by the age at which patients could be recruited.

Table 36: Basic demographic details of patients in ARSACS natural history study

		Number (%)
Gender	Male : Female	15 : 11 (57.7 : 42.3)
Age (years)	Mean ± SD	43.4 ± 13.7
	Median	46
	Range	21 - 69
Disease duration	Mean ± SD	28.5 ± 12.9
(years)	Median	28.5
	Range	8 – 56
Ethnicity	White Caucasian	26 (100)
Country of birth	UK	25 (96.2)
	Switzerland	1 (3.8)
Marital status	Single	13 (50)
	Married/in relationship	8 (30.8)
	Divorced/separated/widowed	5 (19.2)
Offspring	Children	13 (50)
	No children	13 (50)
Education (ISCED)	1 : Primary school	0
	2 : 'O' level/GCSE	14 (53.8)
	3 : 'A' level	1 (3.8)
	4 : BTEC/NVQ/HND	9 (34.6)
	5 : BSc/MSc	1 (3.8)
	6 : PhD	0
	Not known	1 (3.8)
Employment	Not in employment	20 (76.9)
	In employment	5 (19.2)
	Full time	2 (7.7)
	Part time	3 (11.5)
	Not known	1 (3.8)

Figure 63: Geographical location of UK patients in ARSACS natural history study
Red dots represent the location of individual patients

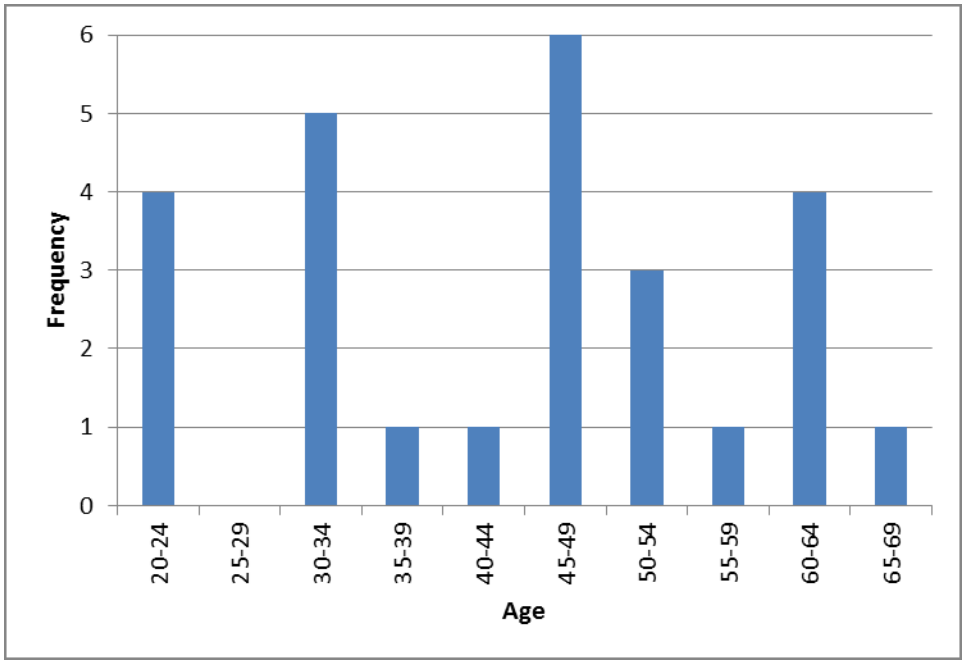


Figure 64: Age at examination of patients in ARSACS natural history study

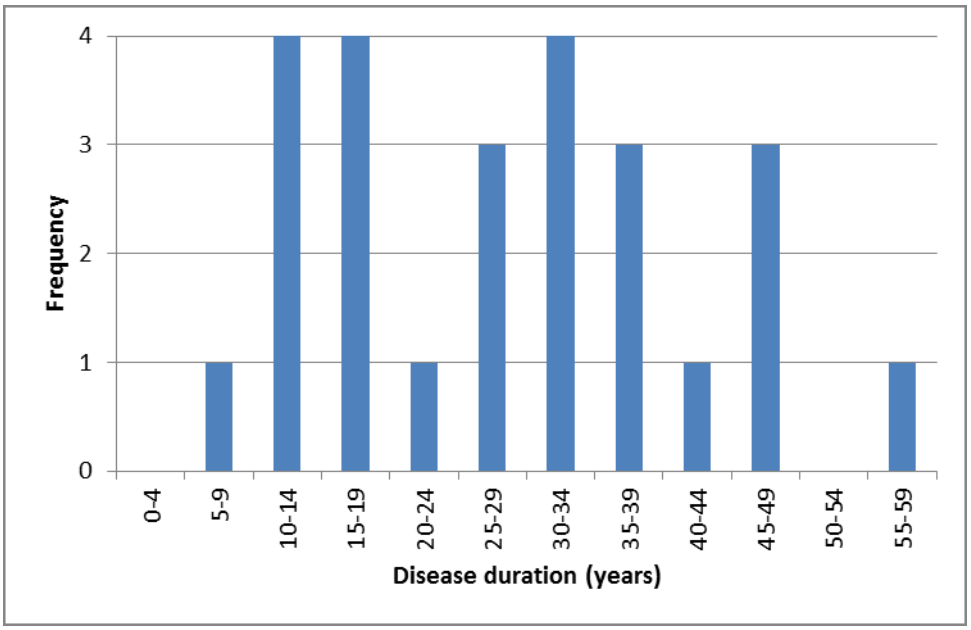
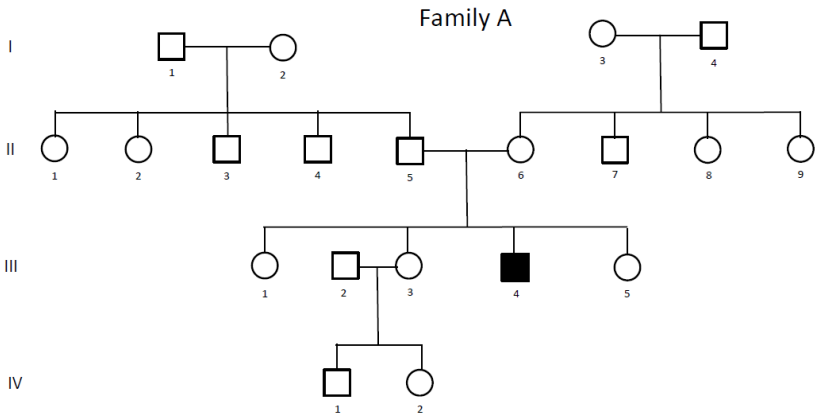


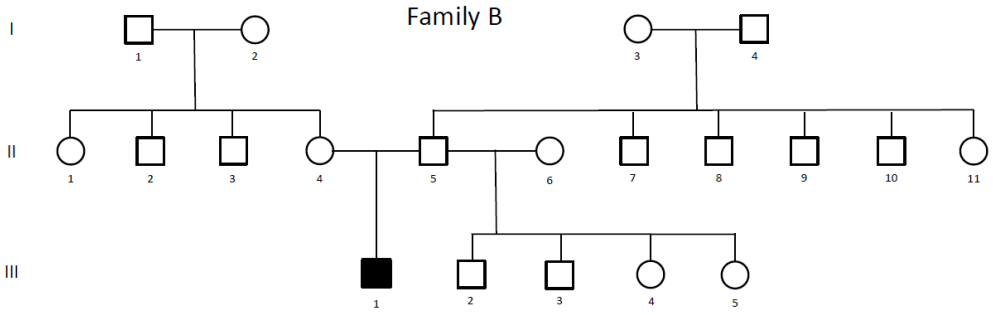
Figure 65: Disease duration at time of examination of patients in ARSACS natural history study

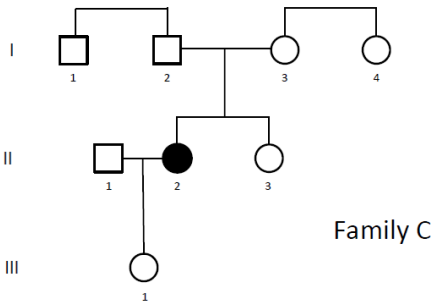
5.3.2 Family Groups

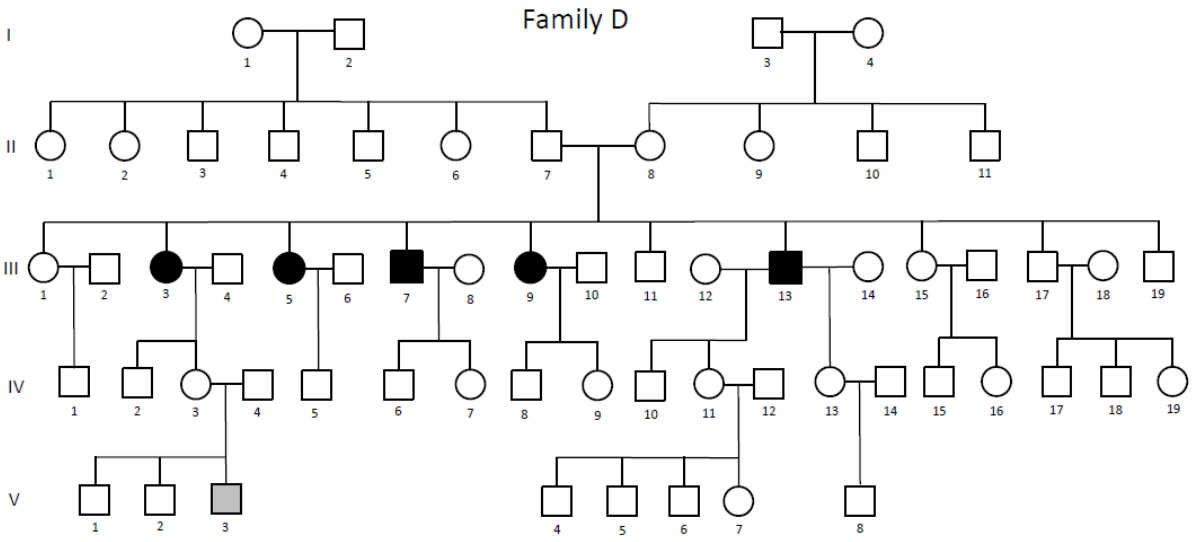
The patients came from 16 families (A-P). Five families (F, J, L, M & P) contained pairs of siblings; one family included 5 siblings (D); one family included a distant cousin as well as two siblings (P). Ten families contained only a single affected individual (A, B, C, E, G, H, I, K, N & O). The genealogical tables of the 16 families are given in Figure 66. Black boxes indicate patients affected with ARSACS (not each of which is in the study).

The meaning of other boxes is described in the text below. Patient numbers, family codes and genetic tree identifiers are summarized in Table 37.

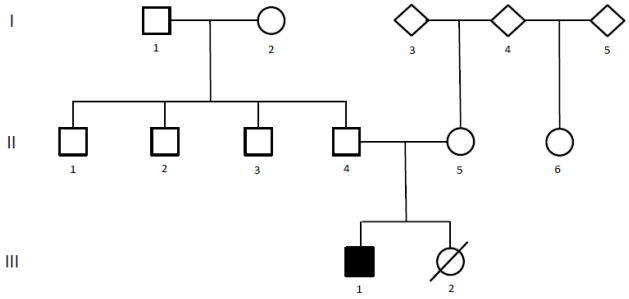


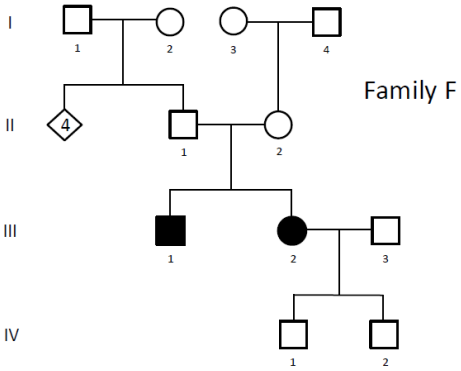






Family E





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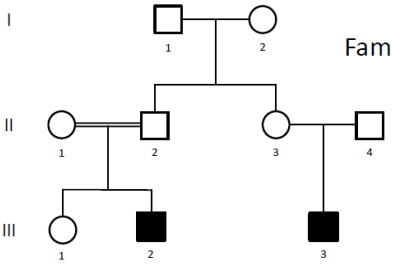

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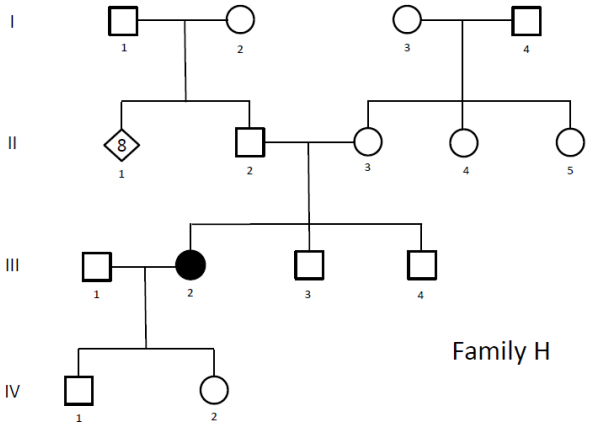

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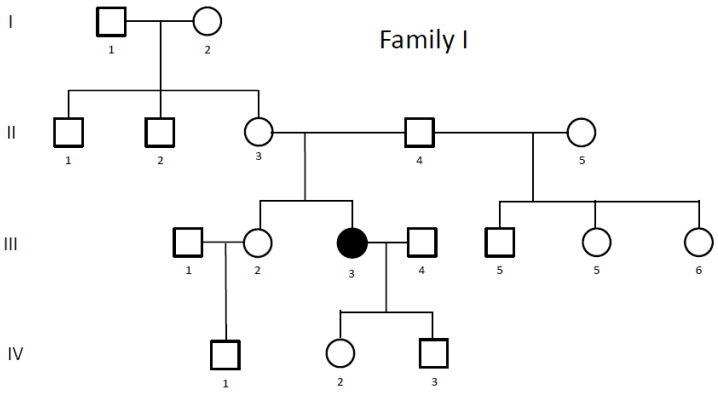
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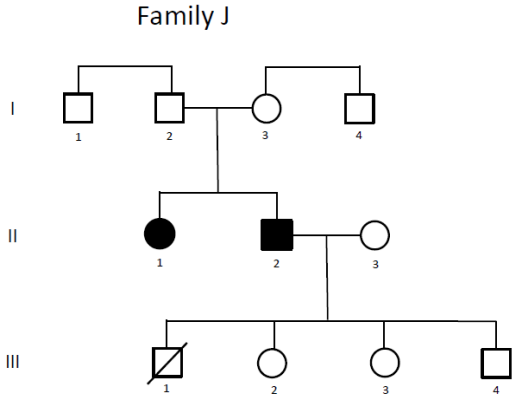
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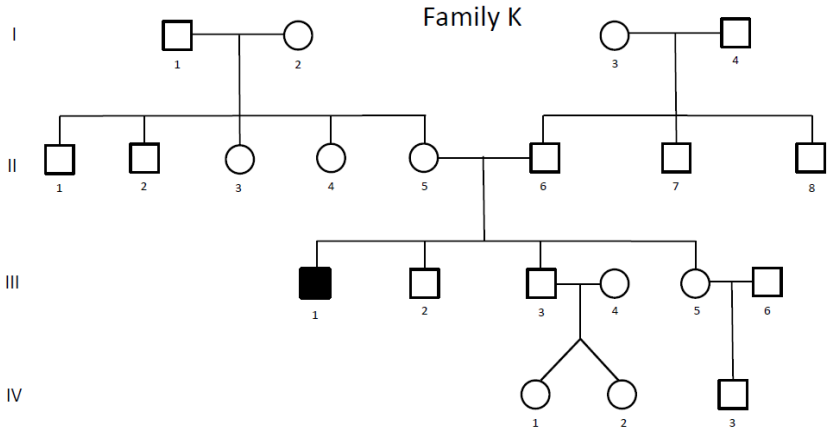
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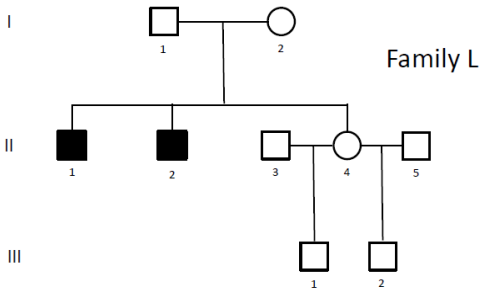
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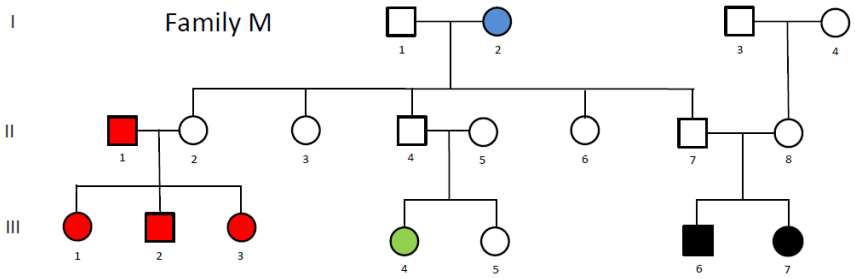
Family M
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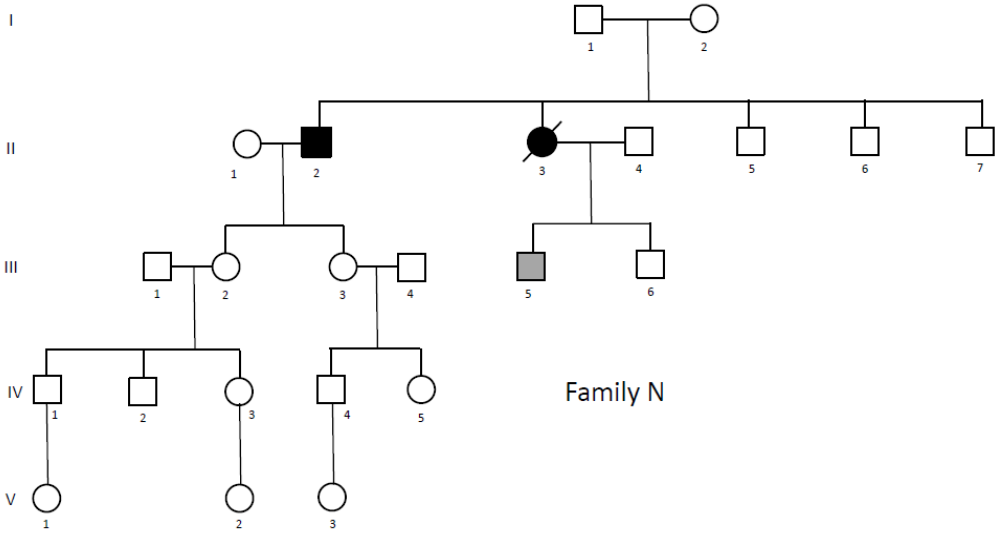
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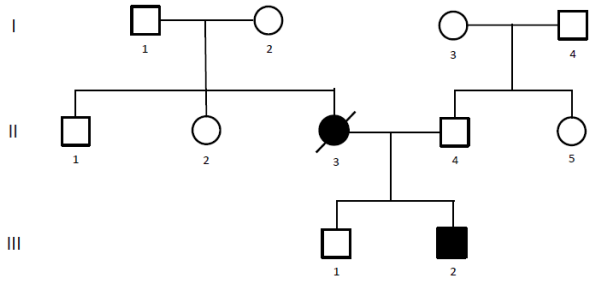
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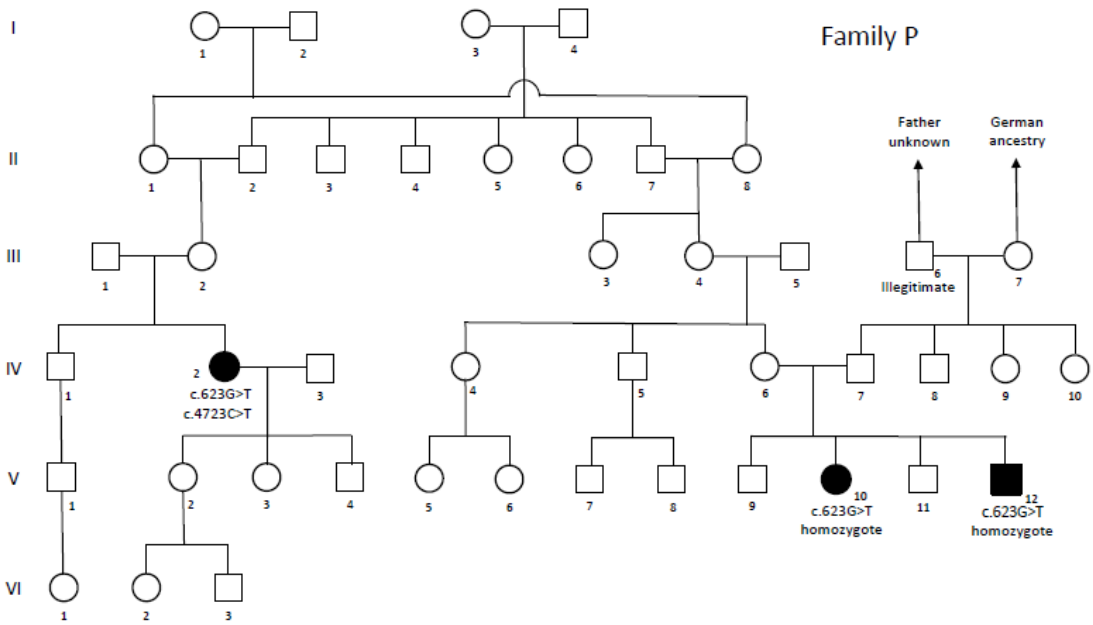

Figure 66: Genealogical tables of families in the ARSACS natural history study. Explanations of the coloured boxes are given in the text.

The clinical features of several of the patients in the study have previously been described to a greater or lesser extent. In particular, patient 1 (Nethisinghe *et al.* 2011) and patient 9 (Stevens *et al.* 2013) have been described in publications from this institution. Patients 18-19 (Pyle *et al.* 2013), 20-21 (Pyle *et al.* 2012) and 18-22 (Yu-Wai-Man *et al.* 2014) were described by Professor Patrick Chinnery's team at the University of Newcastle, and patient 23 was described by Dr Simon Hammans' team at the University of Southampton (Terracciano *et al.* 2010).

In family D, patient V-3 (grey box), the grandchild of affected patient III-3, was said to have an unexplained, progressive neurological condition from early life which may have involved ataxia. Few clinical details were available although he and his mother (patient IV-3) supplied a DNA sample with consent (see Section 5.3.3). In family G, only patient III-2 was seen as part of the study. He disclosed that he had a first cousin (patient III-3) with a very similar early-onset progressive neurological condition causing unsteadiness on walking but also mental retardation. The whole family came from the same small Swiss village but were originally of Calabrian origin. He is presumed to have the same condition clinically, although no DNA was available for genetic confirmation.

Two siblings were seen from family M but the family also included a number of other neurological and genetic conditions. The affected siblings' paternal grandmother

(patient I-2, blue circle) was diagnosed with muscular dystrophy. Their paternal first cousin (patient III-4, green circle) had cystic fibrosis. A further three cousins (patients III-1, III-2 & III-3, red boxes) were diagnosed with Huntington's disease, although this was known to be inherited from an unrelated uncle (patient II-1) and from patient II-1's father (not included in table). In family N, patient II-2 was recruited into the study. His sister (patient II-3) had died but had progressive difficulty in walking with her feet turned in and slurred speech. The onset was said to be before her 30s and possibly in childhood. She was diagnosed clinically with ARSACS by her neurologist. She died in her 60s. Her son (patient III-5, grey box) also has walking problems with the feet turned in and slurred speech with onset in childhood. He had not been diagnosed genetically.

Family O has already been published as an example of pseudo-dominant inheritance of ARSACS (Terracciano et al. 2010). Patient III-2 was recruited into the study. He had delayed motor milestones and a broad-based unsteady gait and slurred speech by the age of 3. He subsequently developed a spastic gait, bilateral pes cavus, limb ataxia, nystagmus, brisk patellar reflexes and absent ankle jerks. His mother (patient II-3) who had already died, had normal early milestones but was noted to be clumsy at school. She suffered further motor deterioration in her teens and was subsequently found to have cerebellar dysarthria, jerky pursuit eye movements, brisk arm and knee reflexes, absent ankle jerks and bilateral Babinski's sign. She was reassessed in her 30s following an episode of diplopia and relapsing weakness. Visual, auditory, and somatosensory evoked potentials showed delayed responses, and CSF examination showed oligoclonal bands. MRI brain showed periventricular white matter abnormalities as well as mild cerebellar, brainstem, and cervical cord atrophy. Genetic analysis showed she was heterozygous for a novel nonsense mutation c.13237C>T (p.Gln4413X) and a 1.5Mb deletion of chromosome 13q12.12 removing 6 genes (*SCGC, SACS, TNFRSF19, MIPEP, SPATA13* & *C1QTNF9*). It was concluded that she had multiple sclerosis in addition to ARSACS. Her son (patient III-2) had a progressive, non-relapsing condition and MRI brain showed pontine tigroid hypointensities and a severely atrophied upper cerebellar vermis but no white matter hyperintensities suggestive of demyelinating disease. Genetically, he had inherited the macrodeletion from his mother and not the

c.13237C>T mutation, but had also inherited a novel nonsense mutation c.2224C>T (p.Arg742X) from his father confirming pseudo-dominant inheritance.

Family P included two siblings who were homozygous for a novel missense variant c.623G>T, and a second cousin once removed who was heterozygous for the c.623G>T variant and a second novel missense variant c.4723C>T (see Figure 66). The cousins were in fact linked genetically via the maternal line of each in two ways, since in generation II, two brothers had married two sisters. It is therefore presumed that each affected individual inherited the c.623G>T variant from one of four common ancestors in generation I (individuals I-1, I-2, I-3 or I-4), and the mothers of each affected individual (individuals III-2 and IV-6) are presumed to be carriers of this variant. If this is the case, patient IV-2's father (individual III-1) must have been a carrier of the c.4723C>T variant. Furthermore, the father of the two affected siblings (individual IV-7) must also be a carrier of the c.623G>T variant. There was no known consanguinuity on this side of the family, but his father (individual III-6) was known to have come from the same small Cornish village, and known to be illegitimate and not to have known who his father was. His wife (individual III-7) was said to have been of German ancestry. A search of the UK General Register Office Index of Births, Deaths and Marriages revealed that no birth certificate was available for individual III-6 in his known name at death. His marriage certificate omitted his father's name and occupation but confirmed that his wife and wife's father had characteristically Germanic names with the occupation of his wife's father recorded as 'Burgomaster'. The family name of individual III-6 is characteristically Cornish. It is therefore presumed that the union of individuals IV-6 and IV-7 was consanguineous and that individual III-6 in some way descended from the same common ancestor of individuals IV-2 and IV-6 who all carried the c.623G>T private variant.

Table 37: Patient numbers, genetic tree identifiers, mutations and basic demographic data of ARSACS patients in natural history study

Patient number	Family	Genetic family tree identifier	Mutation 1	Mutation 2	Sex	Age at exam	Age at onset	Disease duration
1	A	III-4	c1144G>T	c11352_11353dupAA	M	33	0	33
2	B	III-1	c7255_7259delGAGAA	c9956_9957delAA	M	23	5	18
3	C	II-2	c5820_5821delAC	c7162_7163delAC	F	33	1	32
4	D	III-9	c8339T>G;c12416T>C	c11675C>G	F	60	48	12
5	D	III-3	c8339T>G;c12416T>C	c11675C>G	F	62	51	11
6	D	III-5	c8339T>G;c12416T>C	c11675C>G	F	61	43	18
7	D	III-13	c8339T>G;c12416T>C	c11675C>G	M	47	35	12
8	D	III-7	c8339T>G;c12416T>C	c11675C>G	M	60	46	14
9	E	III-1	c5151dupA	c5948C>T; 6392delT	M	40	2	38
10	F	III-1	c9404T>C	c11265_11266delAT	M	45	10	35
11	F	III-2	c9404T>C	c11265_11266delAT	F	39	13	26
12	G	III-2	c6078delT	c6078delT	M	21	2	19
13	H	III-2	c4226_4229delATGA	c9404T>C	F	50	32	18
14	I	III-3	c3149C>A	c4744G>T	F	32	5	27
15	J	II-1	c9404T>C	c12028C>T	F	46	3	43
16	J	II-2	c9404T>C	c12028C>T	M	51	5	46
17	K	III-1	c9956_9957delAA	c10115dupC	M	31	1	30
18	L	II-2	c13048G>T	0.7Mb del 13q12.12	M	47	13	34
19	L	II-1	c13048G>T	0.7Mb del 13q12.13	M	50	1	49
20	M	III-6	c3965_3966delGT	c2076delC	M	46	19	27
21	M	III-7	c3965_3966delGT	c2076delC	F	49	3	46
22	N	II-2	c6781C>A	c1580G>C	M	69	34	35
23	O	III-2	c2224C>T	1.5Mb del 13q12.12	M	24	1	23
24	P	IV-2	c623G>T	c4723C>T	F	57	1	56
25	P	V-10	c623G>T	c623G>T	F	31	1	30
26	P	V-12	c623G>T	c623G>T	M	22	14	8

5.3.3 Genetic Studies

The 26 patients harboured 30 different variants which are predicted to be pathogenic. The genetic variant for each patient is shown in Table 37. The nature of each of the

genetic variants is shown in Table 38 including the predicted amino acid change, predicted consequence to the protein, mutation type and exon site. The LOVD SACS variant ID is given for those variants which have previously been described and registered in the SACSIN database of the Leiden Open Variation Database (LOVD) (https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=SACS). This site aims to record all pathogenic variants published in the *SACS* gene. Patient 9 harbours 3 variants predicted to be pathogenic (c.5151dupA, c.5948C>T and c.6392delT). It was known from a previous study that this patient's unaffected mother carried the c.5948C>T and c.6392delT variants, whilst his unaffected father carried the c.5151dupA variant, showing that c.5948C>T and c.6392delT cosegregate (Stevens et al. 2013). Of note, the c.6392delT variant is a nonsense mutation resulting in frameshift and a premature stop codon after 15 codons, and is therefore obligately pathogenic. The five affected siblings in family D (patients 4,5,6,7 & 8) also harbour 3 variants predicted to be pathogenic (c.8339T>G, c.11675C>G and c.12416T>C). Studies on unaffected first degree relatives of these individuals for the OCT study in Chapter 6 show that c.8339T>G and c.12416T>C cosegregate. Patient V-3 who had an unspecified, early-onset, progressive neurological condition, was not found to have inherited any pathogenic variants. His mother was found to carry the c.11675C>G variant but not the c.8339T>G and c.12416T>C variants. His neurological condition therefore remains unexplained.

The variants seen in this study included two macrodeletions, ten microdeletions, fifteen nucleotide substitutions and three duplications. These gave rise to twenty-one nonsense changes including the direct insertion of a stop codon in six cases, and frameshift changes leading to the premature insertion of a stop codon in a further thirteen cases. There were nine missense changes involving substitution of a different amino acid.

Four of the variants occur in exon 8; all the remainder are in the giant exon 10 where most other published mutations have been found. Sixteen of the variants have previously been published as causing ARSACS. The remainder do not appear as known variants in the LOVD SACSIN database, or the University of California Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&position=

chr13%3A23902915-24007891&hgsid=446324527_b6GeP7MXW8byWKW8v ECMoV2TmRhR), or in the Online Mendelian Inheritance in Man (OMIM) database (http://www.omim.org/entry/604490) or in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). Five variants are described in the PhD thesis of Dr Sacha Vermeer of the University of Radboud, Nijmegen, Netherlands (Vermeer 2012) which have not been published in peer-reviewed journals. These include four nonsense mutations and one missense variant (c.9404T>C). Nine further variants have never previously been described. These include four nonsense mutations and five missense variants (c.623G>T, c.3149C>A, c.4723C>T, c.8339T>G and c.12416T>C). Of note, Baets *et al* have described a symptomatic missense mutation in the neighbouring nucleotide to the c.4723C>T variant which results in a different amino acid sequence change in the same codon (c.4724G>C/p.Arg1575Pro), supporting the notion that the novel mutation is pathogenic (Baets *et al.* 2010).

The nonsense mutations are considered obligately pathogenic because they involve the premature insertion of a stop codon. Taking the fourteen variants from the Vermeer thesis and the novel variants together, in two cases these occurred by direct substitution of an amino acid residue for a stop codon and in six cases by a frameshift mutation (five deletions and one duplication) which resulted in premature insertion of a stop codon between four and fifty-seven codons later in the DNA sequence. The remaining six (missense) variants involved substitution of one amino acid for another.

Various *in silico* techniques were employed in order to predict pathogenicity in the six novel missense variants, described in 5.2.3. The results are summarised in Table 39. All the variants were absent from the 1000 Genomes and dbSNP databases except c.8339T>G which was present with minor allele frequencies (MAF) of 0.0009 and 0.0008 respectively. Both this variant and the c.9404T>C variant were present in the ESP database with MAFs of 0.0035 and 0.00008 respectively. The GERP scores for all the variants were positive, indicating conservation, with four of the values greater than 5, indicating very strong conservation. The lowest was for the c.623G>T variant with a value of 1.27. The lowest PhastCons score was 0.964, with four of the values 1.000, indicating very strong conservation. All the PhyloP scores were positive, indicating conservation, five of which were greater than 4, and two of which were greater than 6,

indicating very strong conservation. The lowest value was for the c.4723C>T variant with a value of 1.072. Multiple sequence alignments for homologous genes from vertebrate species are shown in Table 40.

All the Grantham scores were greater than 100 corresponding with a moderately radical change in amino acid properties; the value for the c.8339T>G variant in which a phenylalanine residue is changed to a cysteine residue, was 205 representing a radical change in amino acid properties. Four of the SIFT scores were less than 0.05 resulting in a prediction of pathogenicity. The c.8339T>G variant had a SIFT score just above this threshold resulting in a prediction of non-pathogenicity. The c.12416T>C variant had a SIFT value of 0.38 placing it clearly in the tolerated category. Four of the variants were predicted by the PolyPhen2 program to be probably damaging with scores of 0.941 or more. The c.3149C>A variant had a PolyPhen2 score of 0.184 which the program predicts to be benign. The Mutation Tasting program which integrates a panoply of different tests representing the above categories, predicts all the variants to be disease-causing, with all the probabilities greater than 0.9366; three of the variants had probabilities of 0.9999 indicating extreme confidence in the prediction.

Table 38: *SACS* gene variants seen in ARSACS patients in natural history study

Variant[a]	Predicted amino acid change[b]	Mutation type	Predicted consequence	Exon	Patient(s)	Reference	LOVD ID
0.7Mb del 13q12.12	-	Macrodeletion	-	-	18,19	(Pyle et al. 2013)	SACS00048
1.5Mb del 13q12.12	-	Macrodeletion	-	-	23	(Terracciano et al. 2010)	SACS00048
c.1144G>T	p.Glu401X	Nonsense substitution	Premature stop codon	8	1	(Vermeer 2012, Nethisinghe et al. 2011)	-
c.1580C>G	p.Ser527X	Nonsense substitution	Premature stop codon	8	22	(Yu-Wai-Man et al. 2014)	SACS00188
c.2076delC	p.Thr692Thr fs*22	Nonsense deletion	Premature stop codon	8	20,21	(Pyle et al. 2012, Yu-Wai-Man et al. 2014)	SACS00184
c.2224C>T	P.Arg742X	Nonsense substitution	Premature stop codon	10	23	(Terracciano et al. 2010)	SACS00061
c.3965_3966delGT	p.Gly1322Val fs*23	Nonsense deletion	Premature stop codon	10	20,21	(Pyle et al. 2012, Yu-Wai-Man et al. 2014)	SACS00185
c.4744G>T	p.Asp1582Asn	Missense substitution	Amino acid substitution	10	14	(Thiffault *et al.* 2013)	SACS00089
c.5151dupA	p.Ser1718Ile fs*20	Nonsense insertion	Premature stop codon	10	9	(Stevens et al. 2013)	SACS00127
c.5948C>T [c]	p.Ser1983Phe	Missense subsitution	Amino acid substitution	10	9	(Stevens et al. 2013)	SACS00129
c.6392delT [c]	p.Phe2131Ser fs*15	Nonsense deletion	Premature stop codon	10	9	(Stevens et al. 2013)	SACS00128
c.6781C>A	p.Leu2261Ile	Missense substitution	Amino acid substitution	10	22	(Yu-Wai-Man et al. 2014)	SACS00189
c.7255_7259delGAGAA	p.Glu2419Phe fs*10	Nonsense deletion	Premature stop codon	10	2	(Prodi *et al.* 2013)	SACS00135
c.11265_11266delAT	p.Ile3755Met fs*9	Nonsense deletion	Premature stop codon	10	10,11	(Baets et al. 2010)	SACS00074
c.11352_11353dupAA	p.Arg3785Lys fs*16	Nonsense insertion	Premature stop codon	10	1	(Vermeer 2012, Nethisinghe et al. 2011)	-

c.13048G>T	p.Glu4350X	Nonsense substitution	Premature stop codon	10	18,19	(Pyle et al. 2013, Yu-Wai-Man et al. 2014)	SACS00120
c.5820_5821delAC	p.Thr1941Leu fs*10	Nonsense deletion	Premature stop codon	10	3	(Vermeer 2012)	-
c.7162_7163delAC	p.Thr2388Arg fs*11	Nonsense deletion	Premature stop codon	10	3	(Vermeer 2012)	-
c.9404T>C	p.Leu3153Ser	Missense substitution	Amino acid substitution	10	10,11,13,15,16	(Vermeer 2012)	-
c.9956_9957delAA	p.Lys3319Ser fs*57	Nonsense deletion	Premature stop codon	10	2,17	(Vermeer 2012)	-
c.11675C>G	p.Ser3892X	Nonsense substitution	Premature stop codon	10	4,5,6,7,8	(Vermeer 2012)	-
c.623G>T	p.Ser208Ile	Missense substitution	Amino acid substitution	8	24,25,26	Novel	-
c.3149C>A	p.Ala1050Asp	Missense substitution	Amino acid substitution	10	14	Novel	-
c.4226_4229delATGA	p.Asn1409Thr fs*19	Nonsense deletion	Premature stop codon	10	13	Novel	-
c.4723C>T	p.Arg1575Trp	Missense substitution	Amino acid substitution	10	24	Novel	-
c.6078delT	p.Ala2026Ala fs*8	Nonsense deletion	Premature stop codon	10	12	Novel	-
c.8339T>G [d]	p.Phe2780Cys	Missense substitution	Amino acid substitution	10	4,5,6,7,8	Novel	-
c.10115dupC	p.Ser3372Ser fs*4	Nonsense duplication	Premature stop codon	10	17	Novel	-
c.12028C>T	p.Gln4010X	Nonsense substitution	Premature stop codon	10	15,16	Novel	-
c.12416T>C [d]	p.Leu4139Ser	Missense substitution	Amino acid substitution	10	4,5,6,7,8	Novel	-

[a] NM_014363.4 [b] NP_055178.3 [c] These variants cosegregate in family E [d] These variants cosegregate in family D

Table 39: *In silico* pathogenicity predictions for *SACS* gene missense variants in ARSACS natural history study

			Presence in Variant Databases			DNA Sequence Conservation Data			Physico-chemical Properties of Amino Acid Substitution		Combined Sequence Conservation & Amino Acid Properties		Sequence Conservation & Effect on Protein Structure		Multifactorial Assessment	
Variant	**Protein change**	**Position[a] (GRCh37)**	**1000 Genomes[b]**	**ESP[c]**	**dbSNP[d]**	**GERP[e]**	**PhastCons[f]**	**PhyloP[g]**	**Grantham score[h]**	**Grantham prediction[i]**	**SIFT score[j]**	**SIFT prediction[j]**	**PolyPhen2 score[k]**	**PolyPhen2 prediction (HumVar)[k]**	**Mutation Tasting prediction[l]**	**Mutation Tasting probability[l]**
c.623G>T	p.Ser208Ile	13:23932576	No	No	No	1.27	1.000	6.311	142	Moderately radical	0.00	Damaging	0.998	Probably damaging	Disease causing	0.9999
c.3149C>A	p.Ala1050Asp	13:23914866	No	No	No	5.96	1.000	6.315	126	Moderately radical	0.01	Damaging	0.184	Benign	Disease causing	0.9999
c.4723C>T	p.Arg1575Trp	13:23913292	No	No	No	4.44	1.000	1.072	101	Moderately radical	0.01	Damaging	0.946	Probably damaging	Disease causing	0.9973
c.8339T>G	p.Phe2780Cys	13:23909676	0.0009 (2)[m]	0.0035 (46)[m]	0.0008[m]	5.32	1.000	4.684	205	Radical	0.07	Tolerated	0.947	Probably damaging	Disease causing	0.9999
c.9404T>C	p.Leu3153Ser	13:23908611	No	0.00008 (1)[m]	No	5.75	0.964	4.793	145	Moderately radical	0.00	Damaging	0.997	Probably damaging	Disease causing	0.9366
c.12416T>C	p.Leu4139Ser	13:23905599	No	No	No	5.30	0.998	4.946	145	Moderately radical	0.38	Tolerated	0.941	Probably damaging	Disease causing	0.9986

[a] UCSC Genome Browser, https://genome-euro.ucsc.edu/cgi-bin/hgGateway?redirect=manual&source=genome.ucsc.edu

[b] 1000 Genomes Project (phase 1, version 3), https://secure.ngrl.org.uk/1kg_querytool/ (accessed via the National Genetics Reference Laboratory (NGRL), Manchester, UK, http://www.ngrl.org.uk/Manchester/page/1000-genomes-dataset).

[c] Exome Sequencing Project (ESP), National Heart, Lung and Blood Institute (NHLBI) Seattle, USA, http://evs.gs.washington.edu/EVS/

[d] Database of Single Nucleotide polymorphisms (dbSNP), National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/projects/SNP/

[e] Genome Evolutionary Rate Profiling (GERP), http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html (calculated using UCSC Genome Browser, http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&position=chr13%3A23902915-24007891&hgsid=447609615_9t6hmVTUgKfvup5vkmGmQx4vcqqP)
[f] Phylogenetic Analysis with Space/Time models Conservation Program (PhastCons) (calculated using Mutation Taster, http://www.mutationtaster.org/ which uses values precomputed by the UCSC Genome Browser[a] comparing MSAs from a phylogeny of 46 vertebrate species)
[g] Phylogenetic p-values (PhyloP) (calculated using Mutation Taster, http://www.mutationtaster.org/ which uses values precomputed by the UCSC Genome Browser[a] comparing MSAs from a phylogeny of 46 vertebrate species)
[h] Granthan R. (1974) Amino Acid Difference Formula to Help Explain Protein Evolution. *Science.* **185**, 862-864.
[i] Li W.H., Wu C.I., and Luo C.C. (1984) Non-randomness of Point Mutation as Reflected in Nucleotide Substitutions in Pseudogenes and its Evolutionary Implications. *J. Mol. Evol*. **21**, 58–71.
[j] Sorting Intolerant From Tolerant (SIFT) algorithm, accessed via http://sift.jcvi.org/
[k] Polymorphisms Phenotyping version 2 (PolyPhen2), accessed via http://genetics.bwh.harvard.edu/pph2/
[l] Mutation Taster 2, accessed via http://www.mutationtaster.org/ using *simple_aae* model for substitution of a single amino acid
[m] These values are quoted as minor allele frequency MAF (absolute count)

1000 Genomes Project: The 1000 Genomes Project Consortium (2012) An integrated Map of Genetic Variation from 1,092 Human Genomes. *Nat.* **491**, 56-65.
ESP: Fu W., O'Connor T.D., Jun G., Kang H.M., Abecasis G., Leal S.M., et al. (2013) Analysis of 6,515 Exomes Reveals the Recent Origin of Most Human Protein-Coding Variants. *Nat.* **493**, 216-220.
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GERP: Cooper G.M., Stone E.A., Asimenos G., NISC Comparative Sequencing Program, Green E.D., Batzoglou S., Sidow A. (2005) Distribution and Intensity of Constraint in Mammalian Genomic Sequence. *Genome Res*. **15**, 901-913.
PhastCons: Siepel A., Bejerano G., Pedersen J.S., Hinrichs A.S., Hou M., Rosenbloom K., Clawson H., *et al*. (2005) Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res*. **15**, 1034-1050.
PhyloP: Siepel A., Pollard K.S. & Haussler D. (2006) New Methods for Detecting Lineage-Specific Selection. *In: Research in Computational Molecular Biology*. **3909**, 190-205.
Grantham Score: Granthan R. (1974) Amino Acid Difference Formula to Help Explain Protein Evolution. *Science.* **185**, 862-864.
SIFT: Ng P.C., and Henikoff S. (2003) SIFT - Predicting Amino Acid Changes that Affect Protein Function. *Nuc. Ac. Res*. **31(13)**, 3812-3814.
PolyPhen2: Adzhubei I.A., Schmidt S., Peshkin L., Ramensky V.E., Gerasimova A., Bork P., Kondrashov A.S., Sunyaev S.R. (2010) A Method and Server for Predicting Damaging Missense Mutations. *Nat. Methods* **7(4),** 248-249.
Mutation Taster: Schwarz J.M., Cooper D.N., Schuelke M., Seelow D. (2014) MutationTaster2: mutation prediction for the deep-sequencing age. *Nat. Methods.***11(4),** 361-362.

Table 40: Multiple sequence alignments generated by the Mutation Taster program

Species: *Homo sapiens* – Human; *Pan troglodytes* – Common chimpanzee; *Macaca mulatta* – Rhesus macaque; *Felis catus* – Domestic cat; *Mus musculus* – House mouse; *Gallus gallus* – Chicken; *Takifugu rubripes* – Japanese pufferfish; *Danio rerio* - Zebrafish

c.623G>T

Species	Match	Gene	aa	Alignment
Human wt			77714	T T G T A T C T T T A G T G G T G A C C A A A T
Human mutated	not conserved		77714	t t g t a t c t t t a **t** t g g t g a c c a a a
P troglodytes	all identical	ENSPTRG00000005703	21156	t t g t a t c t t t a **g** t g g t g a c c a a a
M mulatta	all identical	ENSMMUG00000001292	23295	t t g t a t c t t t a **g** t g g t g a c c a g a
F catus	all identical	ENSFCAG00000012121	14091	c t g t a t c t t t a **g** t g g t g a c c a g a
M musculus	all identical	ENSMUSG00000048279	54660	t t g t a t c t t t a **g** t g g t g a c c a g a
G gallus	all identical	ENSGALG00000017120	14530	t a g t a t t t t c a **g** t g g t g a c c a a a
T rubripes	no alignment	ENSTRUG00000000662		

c.3149C>A

Species	Match	Gene	aa	Alignment
Human wt			92976	G A T G G T A T C A G C T G G T G A A C T C T T
Human mutated	not conserved		92976	g a t g g t a t c a g **a** t g g t g a a c t c t
P troglodytes	all identical	ENSPTRG00000005703	36391	g a t g g t a t c a g **c** t g g t g a a c t c t
M mulatta	all identical	ENSMMUG00000001292	38818	g a t g g t a t c a g **c** t g g c g a a c t c t
F catus	all identical	ENSFCAG00000012121	25231	g a t g g t g t c a g **c** c g g c g a a c t c t
M musculus	all identical	ENSMUSG00000048279	67205	g t g g t a g c a g **c** t g g t g a t c t c t
G gallus	no alignment	ENSGALG00000017120		

c.4723C>T

Species	Match	Gene	aa	Alignment
Human wt			94550	T C A T T A T G A G T C G G G A A T T C A T G A
Human mutated	not conserved		94550	t c a t t a t g a g t **t** g g g a a t t c a t g
P troglodytes	all identical	ENSPTRG00000005703	37965	t c a t t a t g a g t **c** g g g a a t t c a t g
M mulatta	all identical	ENSMMUG00000001292	40392	t c a t t a t g a g t **c** g g g a a t t c a t g
F catus	all identical	ENSFCAG00000012121	28061	t c a t t a t g a g t **c** g a g a a t t c a t g
M musculus	not conserved	ENSMUSG00000048279	68779	t c a t t a t g a g c **a** g a g a a t t t a t g
G gallus	all identical	ENSGALG00000017120	24338	a t t t t g a g c **c** g g g a a t t c a t g
T rubripes	not conserved	ENSTRUG00000000662	10641	t c a t a a t g a g c **a** g a g a g t t c a t g
D rerio	no alignment	ENSDARG00000091042		

c.8339T>G

Species	Match	Gene	aa	Alignment
Human wt			98166	A A G G A A A C A A T T T C A T G C A T C T G T
Human mutated	not conserved		98166	a a g g a a a c a a t **g** t c a t g c a t c t g
P troglodytes	all identical	ENSPTRG00000005703	41581	a a g g a a a c a a t **t** t c a t g c a t c t g
M mulatta	all identical	ENSMMUG00000001292	44008	a a g g a a a c a g t **t** t c a t g c a t c t g
F catus	all identical	ENSFCAG00000012121	31702	a a c a g t **t** t c a c g c a t c t g
M musculus	all identical	ENSMUSG00000048279	72395	a a g g a a g c a a t **t** c c a c g c c t c t g
G gallus	all identical	ENSGALG00000017120	27960	c a a t **t** c c a t g c a t c t g
T rubripes	no alignment	ENSTRUG00000000662	n/a	

c.9404T>C

Species	Match	Gene	aa	Alignment
Human wt			99231	T T T A A A A C T T T T A G T T G A T T A T T G
Human mutated	not conserved		99231	t t t a a a a c t t t **c** a g t t g a t t a t t
P troglodytes	all identical	ENSPTRG00000005703	42646	t t t a a a a c t t t **t** a g t t g a t t a t t
M mulatta	all identical	ENSMMUG00000001292	45463	t t t a a a a c t t t **t** a g t t g a t t a t t
F catus	no alignment	ENSFCAG00000012121	n/a	
M musculus	all identical	ENSMUSG00000048279	73460	t t t a a a a c t t t **t** a g t t g a t t a c t
G gallus	all identical	ENSGALG00000017120	29025	c c t c a a a c t t c **t** g g t t g a c t a t t
T rubripes	no alignment	ENSTRUG00000000662		

c.12416T>C

Species	Match	Gene	aa	Alignment
Human wt			102243	A C T T G A C A G T T T A G G A G T G A A A T A
Human mutated	not conserved		102243	a c t t g a c a g t t **c** a g g a g t g a a a t
P troglodytes	all identical	ENSPTRG00000005703	45658	a c t t g a c a g t t **t** a g g a g t g a a a t
M mulatta	all identical	ENSMMUG00000001292	48475	a c t t g a c a g t t **t** g g g a g t g a a a t
F catus	no alignment	ENSFCAG00000012121	n/a	
M musculus	all identical	ENSMUSG00000048279	76472	g c t t g a c a g t t **t** a g g g g t g a a a t
G gallus	all identical	ENSGALG00000017120	32037	g c t t g a c a g t t **t** a g g g g t a a a g t
T rubripes	all identical	ENSTRUG00000000662	18331	g t t g g a c a a c c **t** c g g g g t t a a a t
D rerio	all identical	ENSDARG00000091042		

5.3.4 Onset & Progression

The mean age at onset amongst the 26 patients in this natural history study was 15.0 years with a range from 0 to 51 years. Twenty-three patients (88.5%) used an aid to walking at least intermittently, from a mean age of 33.2 years. Twenty-two patients (84.6%) used an aid to walking permanently, from a mean age of 39.8 years. Only five patients in the study (19.2%) were permanently wheelchair-bound, which occurred at a mean age of 43.4 years. Further details of these significant milestones in disease progression are given in Table 41.

Table 41: Significant milestones in ARSACS disease progression

		Age (years)
Age at onset	Mean ± SD	15.0 ± 17.4
(*n*=26)	Median	5.0
	Range	0 – 51
Age at intermittent support for walking	Mean ± SD	33.2 ± 16.3
(*n*=23)	Median	28.0
	Range	1 – 61
Age at permanent support for walking	Mean ± SD	39.8 ± 12.0
(*n*=22)	Median	39.0
	Range	20 – 61
Age at wheelchair-bound	Mean ± SD	43.4 ± 16.8
(*n*=5)	Median	43.0
	Range	20 - 67

The range of ages at onset is depicted in Figure 67 showing a clear tendency to onset in the first 5 years of life as had been seen in the original Québecois cases, but with onset up to 51 years of age, which was not described in those original cases but has been seen in cases from other parts of the world. Figure 68 shows the symptoms at onset of patients in the study which are dominated by gait instability and falls.

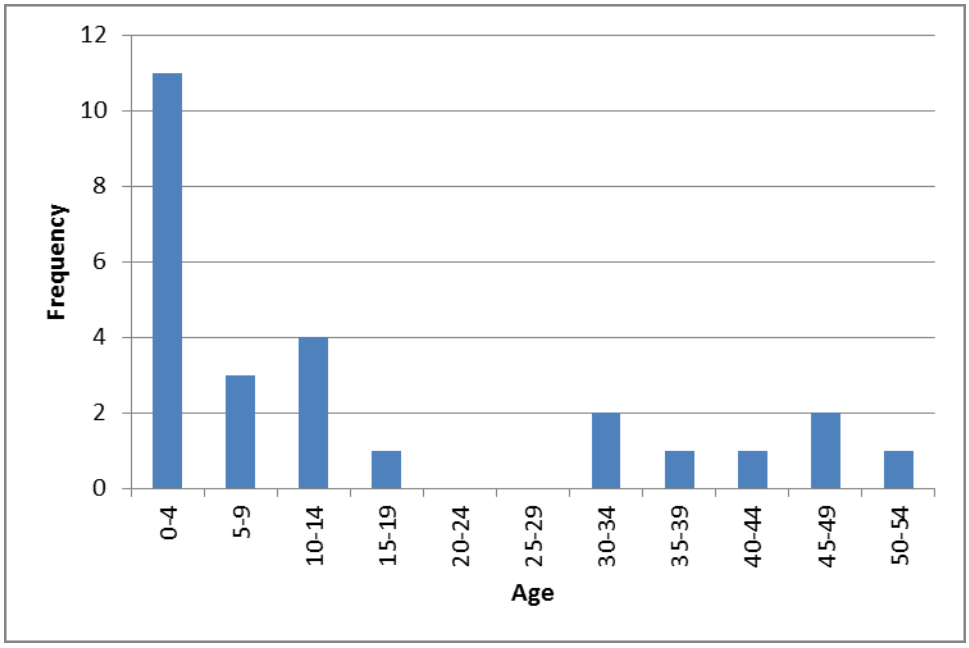

Figure 67: Age at onset of patients in ARSACS natural history study

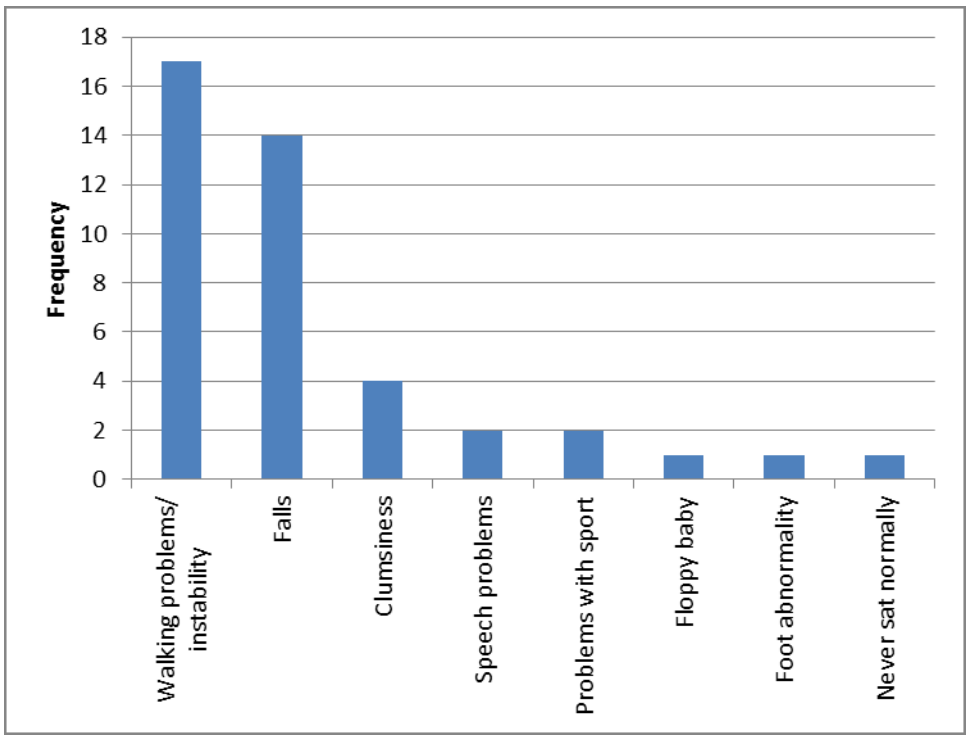

Figure 68: Symptoms at onset in ARSACS patients

The level of disability at examination was classified according to the Spinocerebellar Degeneration Functional Scale (SDFS) as described in Chapter 2 which is based on mobility. The absolute values and descriptions of stages are given in Table 42, and the proportions depicted in Figure 69. Twenty-four of the 26 patients (92.3%) complained of falls. The mean age at onset of falls was 16.6 ± 19.3 years (median 5 yrs; range 1-60).

Table 42: Spinocerebellar Degeneration Functional Score for ARSACS patients

SDFS	Number (%)
1: No functional handicap but signs at examination	0 (0)
2: Mild, able to run, walking unlimited	2 (7.7)
3: Moderate, unable to run, limited walking without aid	4 (15.4)
4: Severe, walking with one stick	6 (23.1)
5: Walking with two sticks	9 (34.6)
6: Unable to walk, requiring wheelchair	5 (19.2)
7: Confined to bed	0 (0)

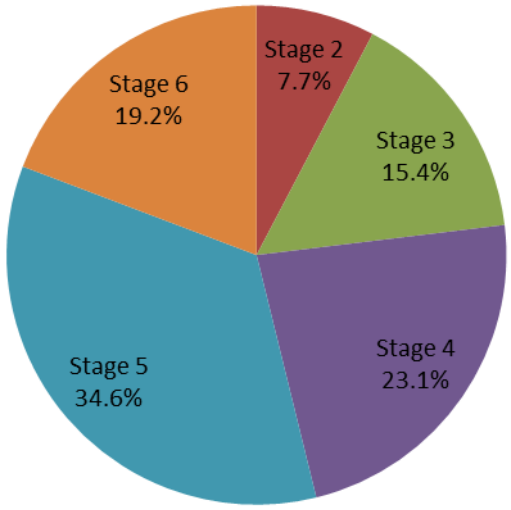

Figure 69: Spinocerebellar Degeneration Functional Score for ARSACS patients

5.3.5 Associated Clinical Features

Nineteen patients (73.1%) complained of visual problems. This included 14 patients (53.8%) which myopia, hypermetropia or astigmatism corrected by spectacles and three (11.5%) with astigmatism. Patient 12 had visual loss attributable to keratoconus. Patient 20 had diplopia corrected by prism spectacles, although no ophthalmoparesis was found on formal examination. Patient 22 had prolonged loss of vision in earlier life as a result of involvement in a gas explosion but had subsequently regained vision. Only two patients (7.7%) complained of oscillopsia directly attributable to ARSACS. Eight patients (30.8%) complained of hearing problems. Two used a hearing aid, in both cases with minimal benefit. Three patients (11.5%) complained of hearing problems in noisy environments.

Five patients (19.2%) complained of headaches or migraines. One patient (3.8%) had

undergone ulnar nerve transposition. Significantly, five patients (19.2%) reported a diagnosis of epilepsy. In one case (patient 25) this was described as 'petit mal' epilepsy in a patient who was found to have a brain cyst on imaging. In one case the epilepsy was described as myoclonic epilepsy (patient 9). Two cases (patients 6 & 8) were siblings in family D who shared the same *SACS* gene mutations.

Twenty-four of the patients (92.3%) had skeletal foot abnormalities. This included 23 (88.5%) with pes cavus, 9 (34.6%) with talipes equinus, 1 (3.8%) with pes planus, 9 (34.6%) with claw toes, and 2 (7.7%) with hammer toes. One patient (3.8%) had spinal scoliosis.

Urological problems such as urinary frequency, incontinence or retention were relatively common affecting 7 patients (26.9%). Five of these were using antimuscarinic medications (tolterodine, trospium chloride, solifenacin & oxybutynin). One was prescribed prophylactic antibiotics (nitrofurantoin). Bowel problems such as chronic constipation were much less common than urological problems, affecting just one patient.

Table 43 details the general medical features seen in the 26 patients with ARSACS. Both patients with diabetes mellitus had type 2 disease. Three patients (patients 5, 9 & 10) had hypertension: all three were aged over 40. Three patients (patients 2, 6 & 10) had hypercholesterolaemia: one of these patients was aged 23, but the others were aged 45 and 61. One patient (patient 10) had all three conditions suggesting an underlying metabolic syndrome. Four patients complained of depression (patients 1, 13, 20 & 23): their ages varied from 24 to 50 but all had disease duration of at least 18 years and two had had essentially lifelong disease. Three patients (patients 11, 14 & 17) had no concomitant medical problems: all were aged in their 30s when seen (mean 34.0, range 31-39).

Patients were taking a wide variety of medications. These included spasticity-related medications in four cases, namely baclofen (patient 4), tizanidine (patient 10), quinine sulphate (patient 16) and gabapentin (patient 21). Two patients took co-enzyme Q_{10} supplements (patients 20 & 21).

Table 43: General medical features seen in ARSACS patients

System	Diagnosis	Number (%)
Metabolic	Diabetes mellitus	2 (7.7)
	Hypothyroidism	2 (7.7)
	Hypercholesterolaemia	3 (11.5)
	Androgen insensitivity	1 (3.8)
Cardiovascular	Hypertension	3 (11.5)
	Supraventricular tachycardia	1 (3.8)
	Viral pericarditis	1 (3.8)
Respiratory	Asthma	3 (11.5)
	Obstructive sleep apnoea	2 (7.7)
Gastrointestinal	Gastritis/acid reflux	2 (7.7)
	Chronic constipation	1 (3.8)
	Coeliac disease	1 (3.8)
	Abdominal hernia	1 (3.8)
	Appendicectomy	1 (3.8)
Urological	Urinary frequency/incontinence/retention	7 (26.9)
	Nephrolithiasis	2 (7.7)
Gynaecological	Uterine fibroids	1 (3.8)
	Hysterectomy (menorrhagia)	1 (3.8)
Ophthalmological	Myopia/hypermetropia/astigmatism	14 (53.8)
	Amblyopia	3 (11.5)
	Keratoconus	1 (3.8)
	Loss of vision after explosion	1 (3.8)
	Blepharitis	1 (3.8)
Otorhinolaryngological	Tonsillectomy/adenoidectomy	2 (7.7)
Rheumatological	Osteoarthritis	2 (7.7)
	Osteoporosis	2 (7.7)
Orthopaedic	Ingrowing toenail	1 (3.8)
	Meniscectomy	1 (3.8)
Dermatological	Psoriasis	1 (3.8)
	Malignant naevus	1 (3.8)
Psychiatric	Depression	4 (15.4)
Neurological	Headaches/migraine	5 (19.2)
	Epilepsy	5 (19.2)
	Brain cyst	1 (3.8)
	Ulnar nerve transposition	1 (3.8)

5.3.6 Activities of Daily Living (ADL)

All 26 patients in the study completed the ADL questionnaire from the Friedreich's Ataxia Rating Scale (FARS). Table 44 shows these results by subscores of the ADL questionnaire. Higher scores represent greater disability. These are depicted graphically in Figure 70. The mean ADL was 15.9±7.7 out of possible total score of 36. The walking and falls subscores were most affected, and the sitting, swallowing and use of cutlery subscores, least.

Table 44: Subscores of the ADL section of the FRDA Rating Scale for ARSACS patients

Score	Speech	Swallow	Use of Cutlery	Dressing	Washing	Falls	Walking	Sitting	Bladder Function	Total
0	5	12	9	4	7	4	0	12	11	64
1	10	7	13	15	10	7	3	11	6	82
2	10	6	4	4	8	3	2	2	3	42
3	1	1	0	2	1	7	16	1	5	34
4	0	0	0	1	0	5	5	0	1	12
Mean	1.3	0.8	0.8	1.3	1.1	2.1	2.9	0.7	1.2	15.9
SD	0.8	0.9	0.7	1.0	0.9	1.4	0.9	0.8	1.3	7.7
Mode	1,2	0	1	1	1	1,3	3	0	0	1

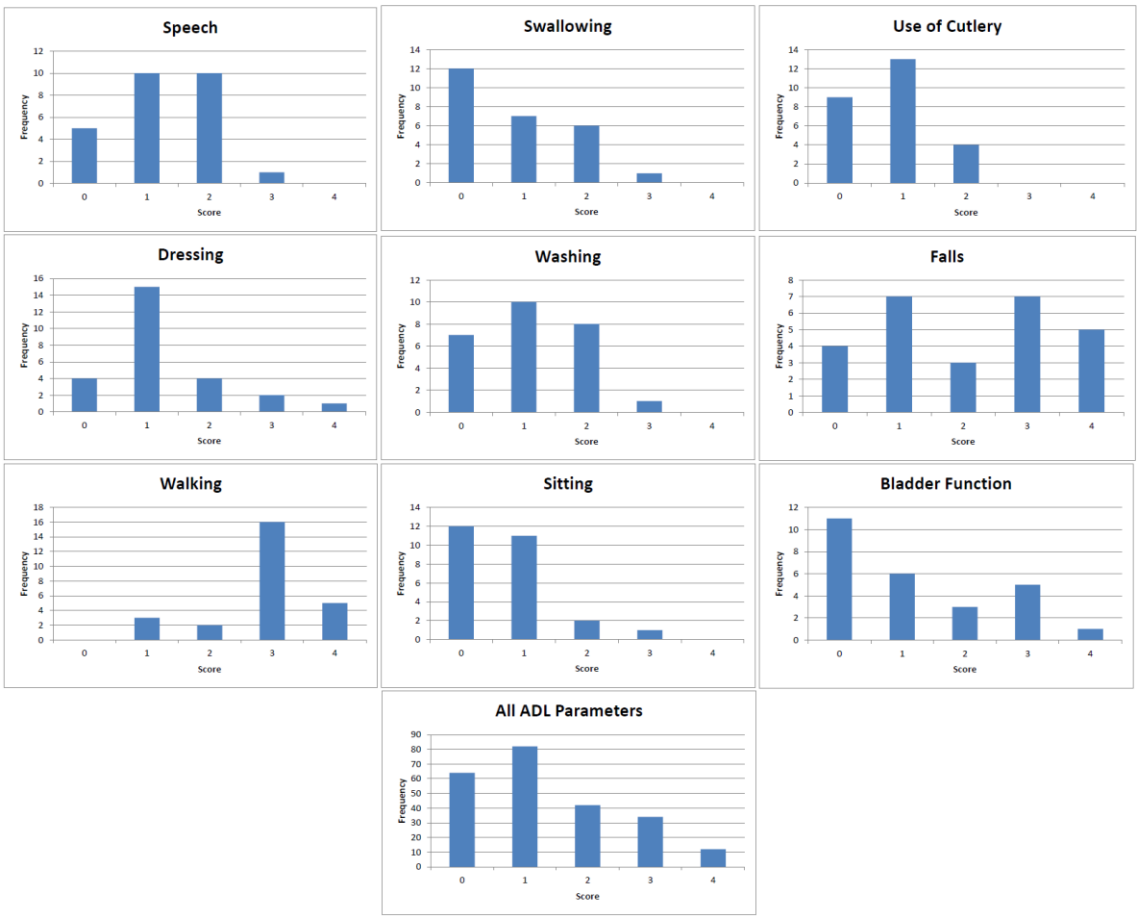

Figure 70: Subscores of the Activities of Daily Living section of the FRDA Rating Scale for ARSACS patients

5.3.7 Scale for the Assessment and Rating of Ataxia (SARA)

The SARA was completed on all 26 ARSACS patients in the natural history study. The results of the subscores are shown in Table 45 and illustrated graphically in Figure 71. The mean SARA score was 16.0±7.7 out of a possible total of 40 points. Higher scores represent more prominent ataxia. The gait and stance subscores were most affected, with the speech, finger chase, nose-finger and fast alternating hand movements subscores, least affected.

Table 45: Subscores of the SARA for ARSACS patients

Score	Gait	Stance	Sitting	Speech	Finger Chase	Nose-Finger Test	Fast Alternating Hand Movements	Heel-Shin Test
0	0	0	12	12	4	3	5	5
0.5	-	-	-	-	4	4	5	2
1	2	2	6	9	18	15	13	7
1.5	-	-	-	-	0	2	0	1
2	3	6	1	3	0	2	0	2
2.5	-	-	-	-	0	0	0	0
3	2	1	3	2	0	0	3	1
3.5	-	-	-	-	0	0	0	0
4	2	3	4	0	0	0	0	8
5	3	4	-	0	-	-	-	-
6	6	0	-	0	-	-	-	-
7	2	-	-	-	-	-	-	-
8	6	-	-	-	-	-	-	-
Mean	5.2	4.2	1.3	0.8	0.8	0.9	0.9	1.9
SD	2.3	1.9	1.5	0.9	0.4	0.5	0.9	1.6
Mode	6,8	2	0	0	1	1	1	4

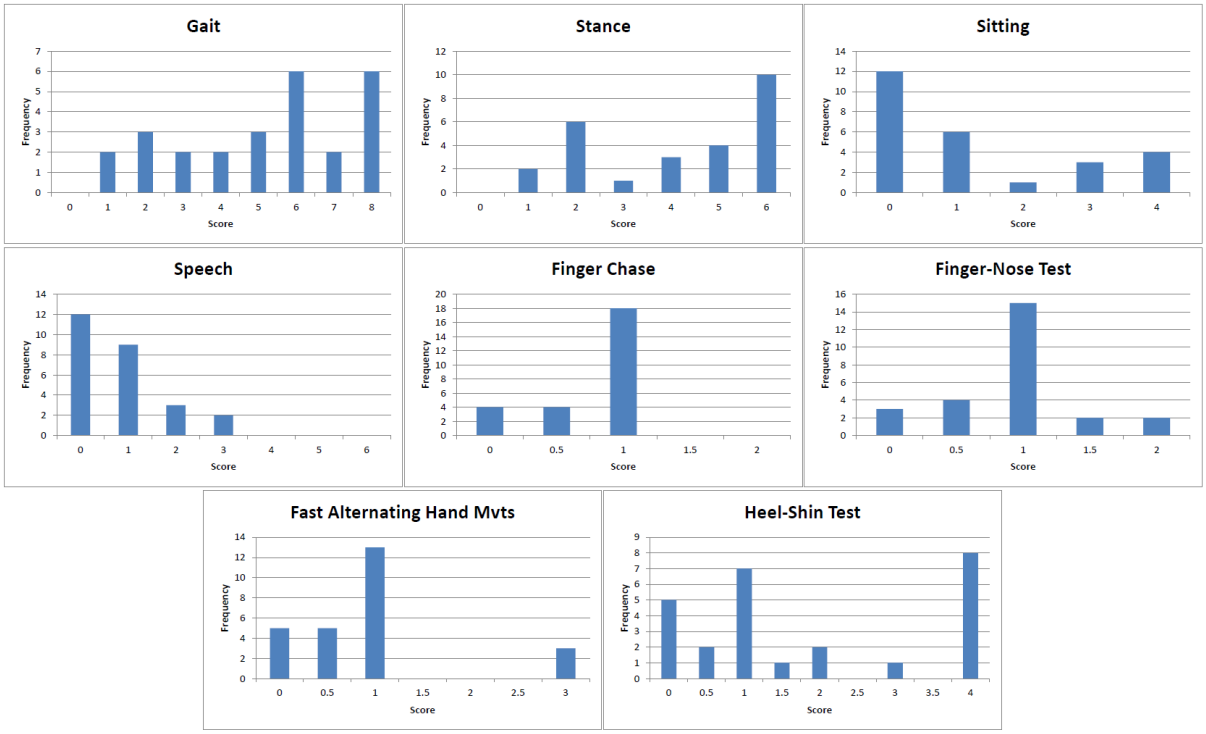

Figure 71: Subscores of the SARA for ARSACS patients

5.3.8 Inventory of Non-Ataxic Symptoms (INAS)

The INAS was assessed for all 26 ARSACS patients in the study. The mean INAS count was 5.7 ± 1.9. Higher scores represent greater disease involvement. The number of patients having each of the INAS count values is shown in Table 46 and shown graphically in Figure 72.

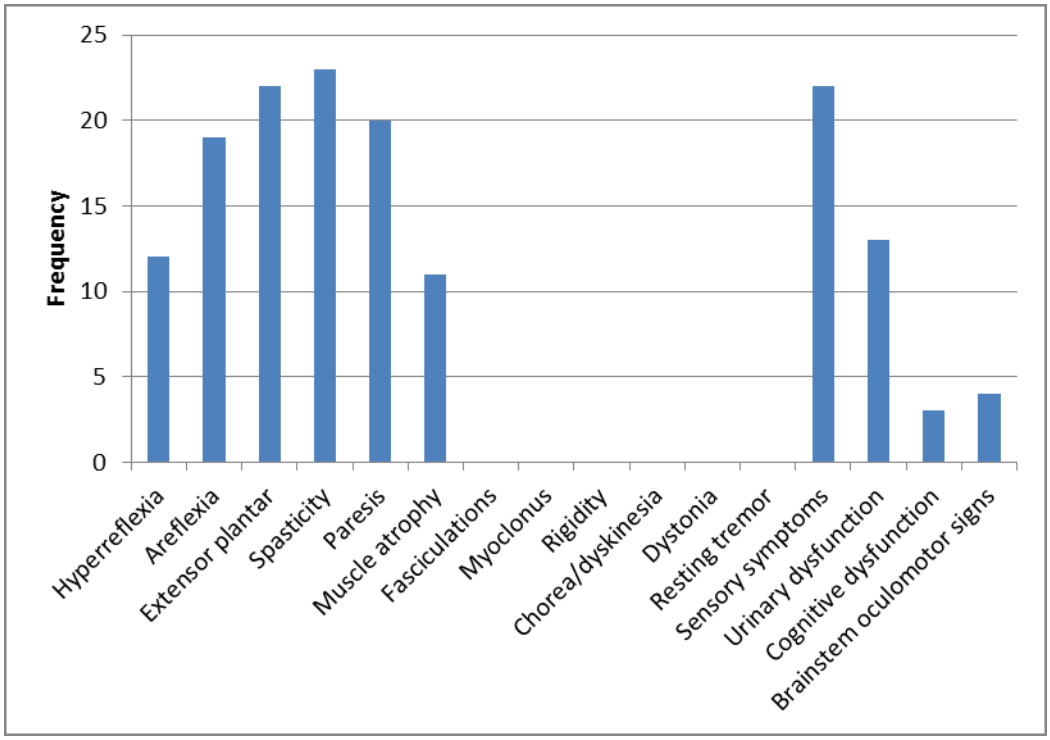

Figure 72: INAS count values for ARSACS patients

The commonest non-ataxic symptom is spasticity, followed by sensory loss, Babinski's sign, weakness and areflexia. Fasciculations, myoclonus, rigidity, dyskinesia, dystonia and resting tremor were not seen, and cognitive dysfunction and brainstem oculomotor signs were only seen in a very low proportion of patients. Table 47 records the base data of non-ataxic signs and symptoms found in the ARSACS patients in the study. Weakness, spasticity & sensory loss are depicted in Figure 73; symptoms reported by patients (diplopia, dysphagia, episodic vertigo, speech problems, problems with handwriting and muscle cramps) are depicted in Figure 74; the pattern of reflexes found at examination as part of the INAS are depicted in Figure 75; ophthalmological features are depicted in Figure 76.

Table 46: INAS count values for ARSACS patients

	No. of patients (%)
Hyperreflexia	12 (46.2)
Areflexia	19 (73.1)
Extensor plantar	22 (84.6)
Spasticity	23 (88.5)
Paresis	20 (76.9)
Muscle atrophy	11 (42.3)
Fasciculations	0 (0)
Myoclonus	0 (0)
Rigidity	0 (0)
Chorea/dyskinesia	0 (0)
Dystonia	0 (0)
Resting tremor	0 (0)
Sensory symptoms	22 (84.6)
Urinary dysfunction	13 (50.0)
Cognitive dysfunction	3 (11.5)
Brainstem oculomotor signs	4 (15.4)

Table 47: Non-ataxic features from the INAS for ARSACS patients [Number (%)]

		None	Mild	Moderate	Severe
Weakness	UL proximal	24 (92.3)	0 (0)	2 (7.7)	0 (0)
	UL distal	14 (53.8)	9 (34.6)	1 (3.8)	2 (7.7)
	LL proximal	10 (38.5)	8 (30.8)	3 (11.5)	3 (11.5)
	LL distal	7 (26.9)	9 (34.6)	5 (19.2)	5 (19.2)
Spasticity	UL	10 (38.5)	15 (57.7)	0 (0)	1 (3.8)
	LL	6 (23.1)	7 (26.9)	8 (30.8)	5 (19.2)
Impaired vibration	LL distal	5 (19.2)	1 (7.7)	5 (19.2)	15 (53.6)

	None	Mild	Moderate	Severe
Diplopia	19 (73.1)	5 (19.2)	1 (3.8)	1 (3.8)
Dysphagia	12 (46.2)	9 (34.6)	3 (11.5)	2 (7.7)
Episodic vertigo	16 (61.5)	3 (11.5)	2 (7.7)	5 (19.2)
Speech problems	8 (30.8)	13 (50.0)	4 (15.4)	1 (3.8)
Handwriting problems	2 (7.7)	9 (34.6)	11 (42.3)	4 (15.4)
Muscle cramps	3 (11.5)	6 (23.1)	5 (19.2)	12 (46.2)

	Normal	Hyperreflexic	Areflexic
Biceps reflex	14 (53.8)	1 (3.8)	11 (42.3)
Patellar reflex	7 (26.9)	12 (46.2)	7 (26.9)
Achilles reflex	8 (30.8)	0 (0)	18 (69.2)
	None	**Unilateral**	**Bilateral**
Extensor Plantar	4 (15.4)	3 (11.5)	19 (73.1)

	Number (%)
Broken smooth pursuits	26 (100)
Square wave jerks on fixation	2 (7.7)
Downbeat nystagmus on fixation	1 (3.8)
Gaze-evoked nystagmus on horizontal testing	25 (96.2)
Gaze-evoked nystagmus on vertical testing	8 (30.8)
Ophthalmoparesis on horizontal gaze	1 (3.8)
Ophthalmoparesis on vertical gaze	1 (3.8)
Slowing of saccades	2 (7.7)
Hypometric saccades	2 (7.7)
Hypermetric saccades	7 (26.9)

UL=upper limb ; LL=lower limb

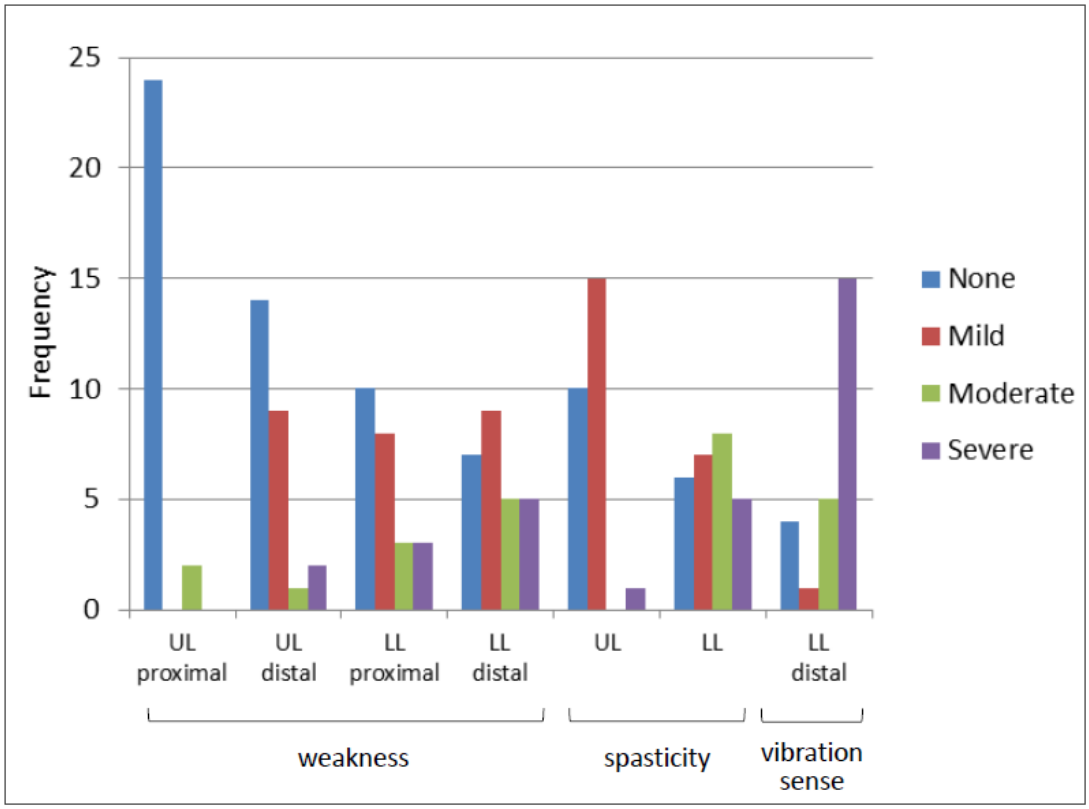

Figure 73: Weakness, spasticity & sensory loss for ARSACS patients as recorded in the INAS

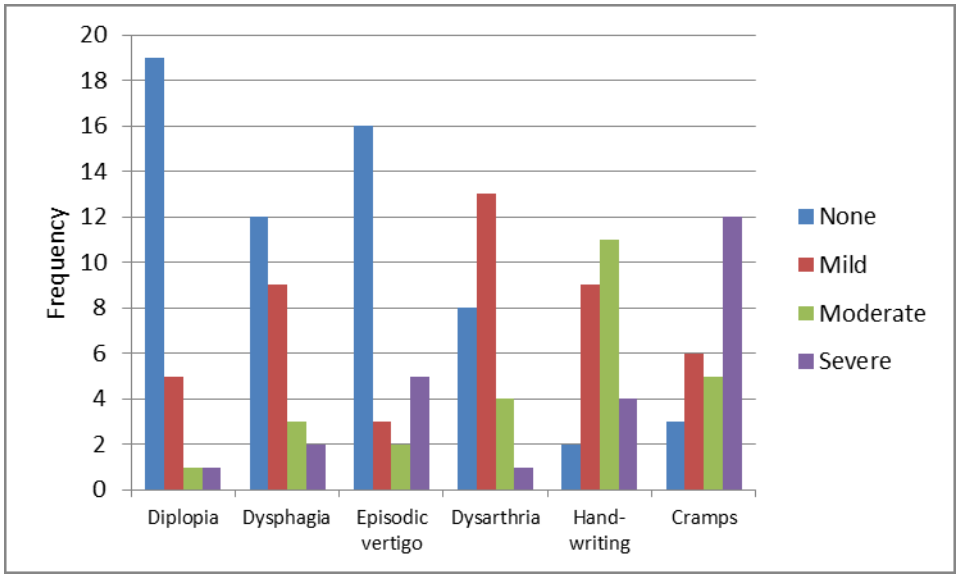

Figure 74: Symptoms reported by ARSACS patients as part of the INAS

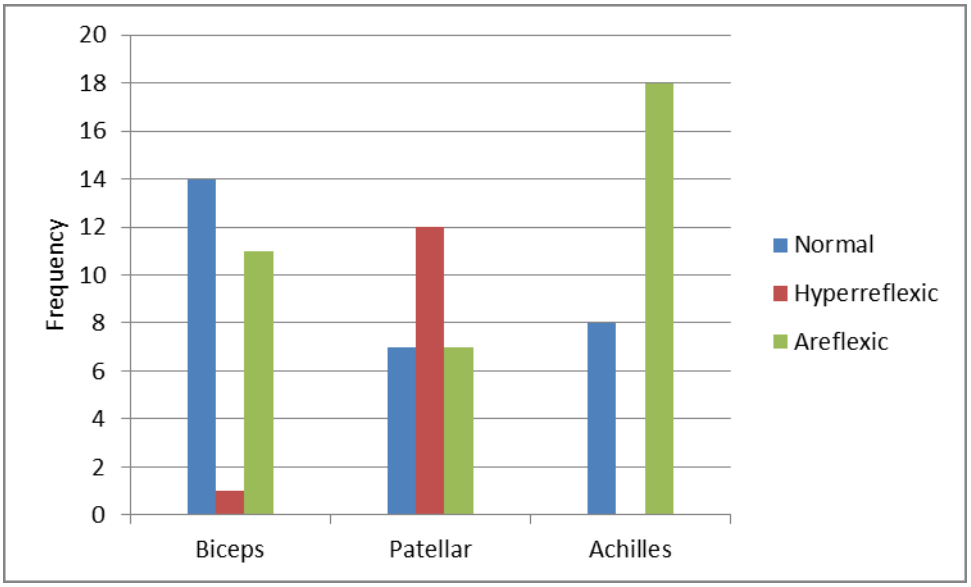

Figure 75: Reflexes for ARSACS patients as recorded in the INAS

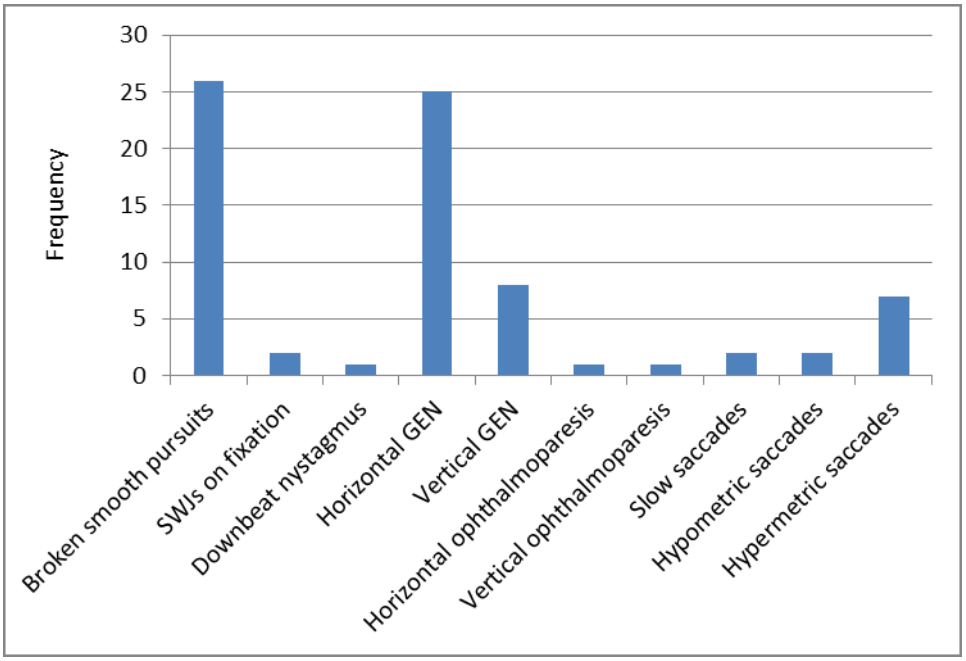

Figure 76: Ophthalmological features of ARSACS patients as recorded in the INAS
GEN=gaze-evoked nystagmus

5.3.9 Spinocerebellar Ataxia Functional Index (SCAFI)

Twenty-five of the 26 ARSACS patients in the natural history study completed the SCAFI although, in common with the EFACTS patients, not all could complete all parts of the rating scale. Eleven patients were able to complete the 8 metre timed walk and 10 were unable to do so. The timed walk was not completed in four cases who were seen on home visits where there was not a flat indoor 8 metre course over which to

time the patient. Therefore, in a clinical setting in which this component of the test could be undertaken, only 52% of the patients could complete it, illustrating a major deficiency in this test. Twenty-three of the 25 patients (92%) were able to undertake the 9-hole peg test. All patients were right hand dominant. All patients could perform the PATA test. The raw data are shown in Table 48 and the calculated Z scores in Table 49. The raw data for all values (before averaging) are shown in Table 48. For the raw data, lower values for the PATA test and higher values for the 8mTW and 9HPT represent greater disease involvement.

Table 48: Raw data from SCAFI for ARSACS patients

Task	No. completing (%)	Mean ± SD	Range
8mTW[1] (s)	11 (52.4)	11.7 ± 3.9	6 - 20
9HPT-D[2] (s)	23 (92.0)	43.5 ± 11.7	25 – 69
9HPT-ND[3] (s)	23 (92.0)	55.3 ± 32.5	26 – 184
9HPT-All[4] (s)	23 (92.0)	49.6 ± 25.0	25 – 184
PATA (/10s)	25 (100)	17.9 ± 5.29	11 - 33

[1]missing values *n*=4
[2]9HPT-D = 9-hole peg test for dominant hand
[3]9HPT-ND = 9-hole peg test for non-dominant hand
[4]9HPT-All = 9-hole peg test for both hands

Table 49: Calculated Z scores from SCAFI for ARSACS patients

	Z-8mTW	Z-9HPT	Z-PATA	SCAFI
Mean	-1.43	-0.26	0.00	-0.03
SD	1.69	1.23	0.96	0.63
Min	-2.99	-3.05	-1.30	-1.35
Max	1.88	1.88	2.11	1.12
n	21	25	25	21

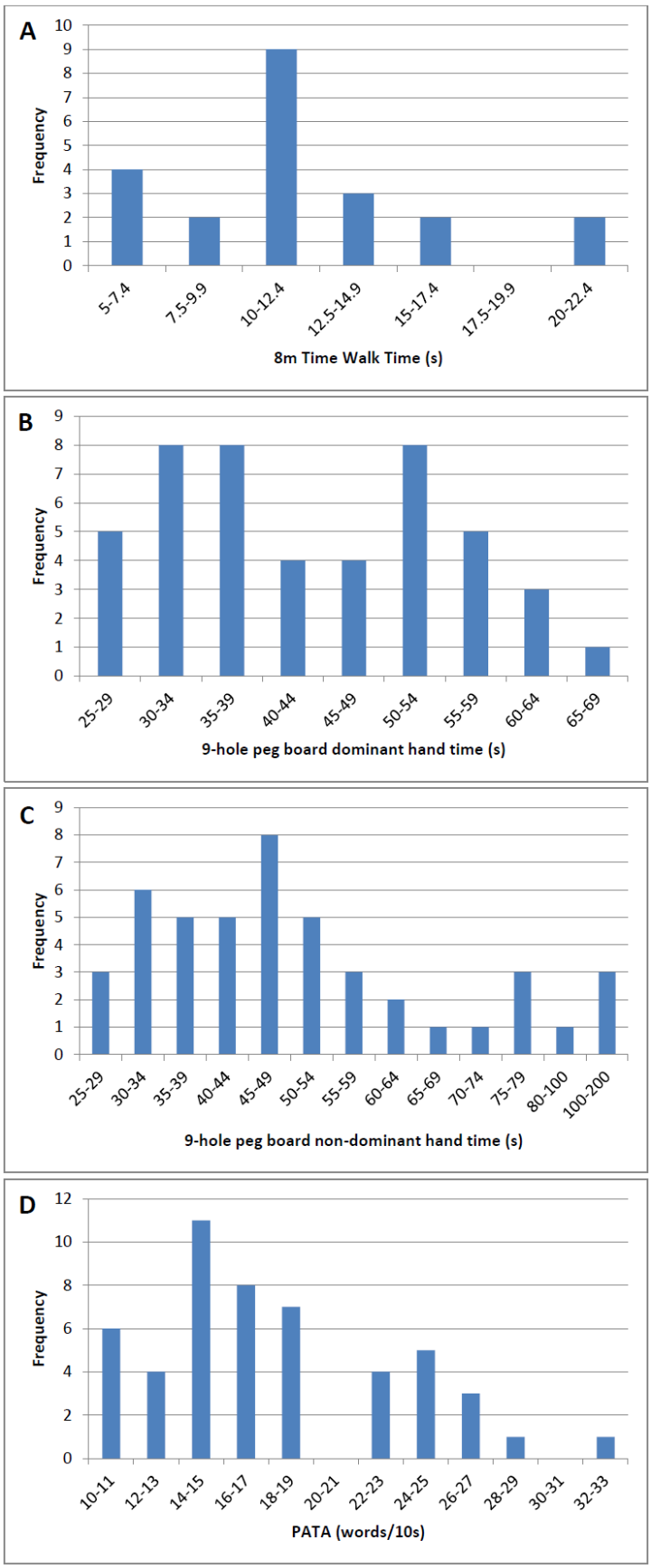

Figure 77: Raw data from SCAFI for ARSACS patients: (A) 8m timed walk; (B) 9-hole peg test for dominant hand; (C) 9-hole peg test for non-dominant hand; (D) PATA test

5.3.10 Structured Neurological Examination (SNE)

All 26 ARSACS patients had the SNE recorded. No patients had ptosis or pupillary abnormalities including a relative afferent pupillary defect (RAPD). Only one patient (patient 9) had any form of ophthalmoparesis; this involved a vertical supranuclear gaze palsy as previously published (Stevens *et al.* 2013). Eight patients (30.8%) wore spectacles. There were no cases of facial weakness or sensory loss, and no examples of masticatory, palatal, sternocleinomastoid or trapezius weakness. One patient reported hearing loss. One patient had tongue atrophy. Six patients (23.1%) showed tongue spasticity and the remainder had normal lingual tone. No patients displayed evidence of parkinsonism.

The range of muscle power found at examination is given in Table 50, with each value summating 52 power measurements (bilateral for the 26 ARSACS patients). It should be noted that the values recorded are on a 7 point scale related to the MRC rating scale as described in Chapter 2. In short, the values up to 3 correspond directly with those of the MRC scale, whilst 4 in the Table below corresponds to 4-/5 in the MRC scale, 5 to 4/5, 6 to 4+/5 and 7 to 5/5. Thus, the mean value of 6.96 for shoulder abduction represents near full power in all patients (in fact, 25 patients had power of 5/5 and one patient 4+/5 bilaterally). The mean values are depicted in Figure 78.

Table 50: Muscle power from SNE in ARSACS patients

	Mean	SD	Mode	Range
Shoulder Abduction	6.96	0.19	7	6-7
Shoulder Adduction	6.96	0.19	7	6-7
Elbow Flexion	6.98	0.14	7	6-7
Elbow Extension	6.98	0.14	7	6-7
Wrist Flexion	6.96	0.19	7	6-7
Wrist Extension	6.92	0.39	7	5-7
Finger Flexion	6.90	0.36	7	5-7
Finger Extension	6.81	0.60	7	4-7
Index Finger Abduction	6.15	1.33	7	3-7
Little Finger Abduction	5.92	1.56	7	2-7
Thumb Abduction	6.23	1.55	7	1-7
UL proximal	6.97	0.17	7	6-7
UL distal	6.56	1.09	7	1-7
Hip Flexion	5.17	2.19	7	0-7
Hip Extension	5.85	2.12	7	0-7
Hip Abduction	6.15	2.05	7	0-7
Hip Adduction	6.15	2.05	7	0-7
Knee Flexion	5.25	2.46	7	0-7
Knee Extension	6.17	1.62	7	1-7
Ankle Flexion	5.12	2.54	7	0-7
Ankle Extension	5.15	2.44	7	0-7
Ankle Inversion	5.00	2.58	7	0-7
Ankle Eversion	4.83	2.63	7	0-7
Toe Flexion	4.28	2.58	7	0-7
Toe Extension	4.44	2.73	7	0-7
LL proximal	5.79	2.12	7	0-7
LL distal	4.81	2.58	7	0-7

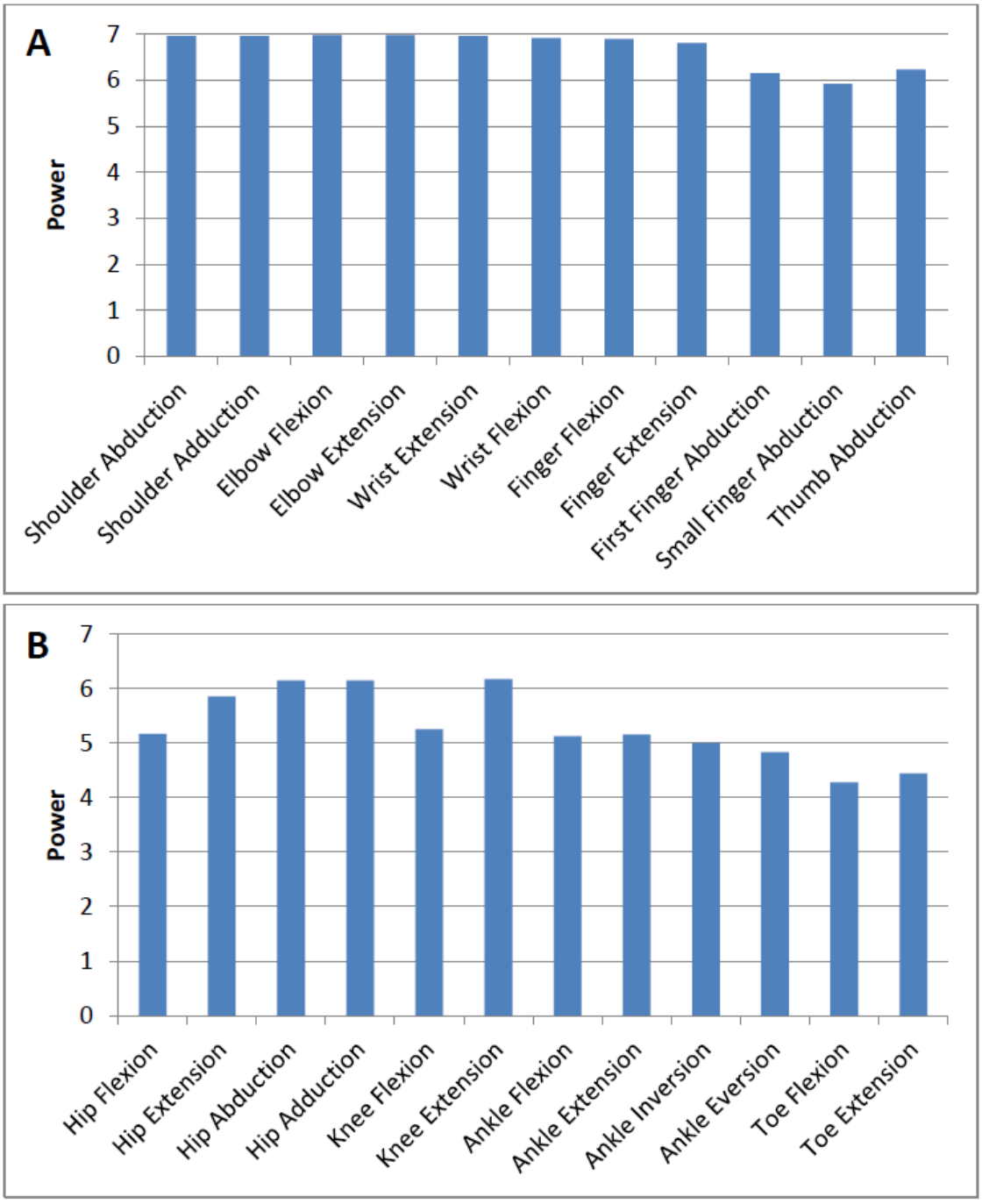

Figure 78: Mean muscle power values from SNE for (A) upper & (B) lower limbs in ARSACS patients.

For an explanation of the scale, see text.

Values for deep tendon reflexes are given in Table 51 which represent bilateral measurements from the 26 patients in the study. The results are recorded on a 6-point scale from absent (0) to 'hyperreflexic with clonus' (5) for the biceps, supinator, triceps, patellar and ankle reflexes. Finger jerks and Hoffman's reflex are recorded as

present or absent. The plantar reflexes are recorded as mute, flexor, extensor or withdrawal (unassessable). The results are further depicted in Figure 79.

Table 51: Deep tendon reflexes from the SNE for ARSACS patients

	Absent	Present only with reinforce-ment	Present but hyporeflexic	Normal	Hyper-reflexic	Hyperreflexic with clonus
Biceps	24 (46.2)	0 (0)	0 (0)	28 (53.8)	0 (0)	0 (0)
Supinator	21 (40.4)	0 (0)	1 (1.9)	30 (57.3)	0 (0)	0 (0)
Triceps	16 (30.8)	0 (0)	3 (5.8)	29 (55.8)	4 (7.7)	0 (0)
Patellar	8 (15.4)	0 (0)	6 (11.5)	19 (36.5)	18 (34.6)	1 (1.9)
Ankle	30 (57.7)	4 (7.7)	4 (7.7)	14 (26.9)	0 (0)	0 (0)

	Absent	Present
Finger jerks	50 (96.2)	2 (3.8)
Hoffman reflex	50 (96.2)	2 (3.8)

	Mute	Flexor	Extensor	Withdrawal
Plantar	6 (11.5)	3 (5.8)	40 (76.9)	3 (5.8)

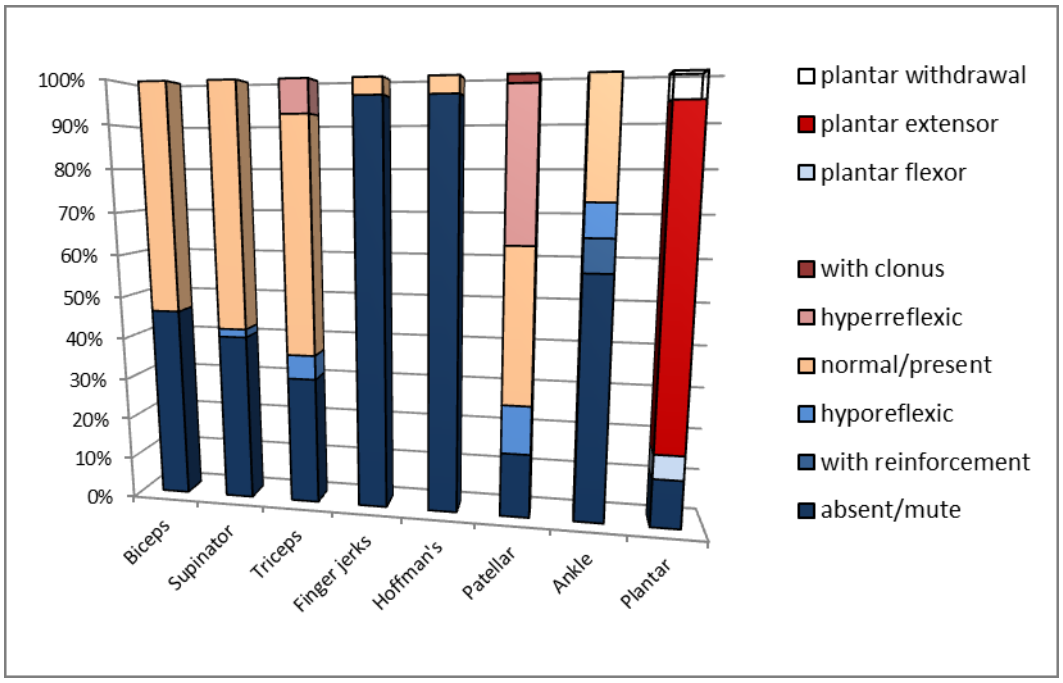

Figure 79: Distribution of deep tendon reflexes from SNE in ARSACS patients

Table 52 gives values for muscle atrophy, muscle tone, sensory loss and skeletal foot abnormalities derived from the SNE for the 26 patients in the ARSACS natural history

study. All values are semi-quantitative and averaged over 52 measurements (bilateral for each value). Muscle atrophy & skeletal foot abnormalities were assessed using a 4-point scale (none, mild, moderate, severe). Muscle tone was evaluated using a 6-point scale from highly flaccid to highly spastic with a further category for other causes of altered tone such as rigidity or Gegenhalten; no patients fell into this category. Sensation was assessed for the three modalities pin prick, proprioception and vibration, using a length-dependent scale described in detail in Chapter 2. This has been analyzed as a continuous variable with zero representing no sensory loss and each point representing a further joint more distal affected. Figure 80 depicts the results for muscle atrophy and spasticity. Figure 81 shows the results for the various modalities of sensation. Figure 82 shows the results for the various types of skeletal foot abnormality seen in ARSACS.

Table 52: Values for muscle atrophy, muscle tone, sensory loss and skeletal foot abnormalities from the SNE for ARSACS patients

		None	Mild	Moderate	Severe
Atrophy	**UL**	38 (73.1)	12 (23.1)	2 (3.8)	0 (0)
	LL	41 (78.8)	7 (13.5)	4 (7.7)	0 (0)

		Highly flaccid	Flaccid	Normal	Spastic	Highly spastic	Other
Tone	**UL**	0 (0)	2 (3.8)	26 (50)	24 (46.2)	0 (0)	0 (0)
	LL	0 (0)	0 (0)	16 (30.8)	12 (23.1)	24 (46.2)	0 (0)

			Mean	SD	Mode	Range
Sensation	**UL**	**Pin prick**	1.02	1.62	0	0-6
		JPS	0	0	0	0
		Vibration	0.15	0.64	0	0-3
	LL	**Pin prick**	1.4	1.56	0	0-5
		JPS	0.92	1.13	0	0-3
		Vibration	1.77	1.37	2	0-4

	Absent	Mild	Moderate	Severe
Pes cavus	7 (13.5)	7 (13.5)	18 (34.6)	20 (38.5)
Pes planus	50 (96.2)	0 (0)	2 (3.8)	0 (0)
Hammer/claw toes	32 (61.5)	0 (0)	14 (26.9)	6 (11.5)
Talipes equinus	34 (65.4)	6 (11.5)	7 (13.5)	5 (9.6)
Talipes varus	36 (69.2)	6 (11.5)	5 (9.6)	5 (9.6)

UL=upper limb; LL=lower limb; JPS=joint position sense

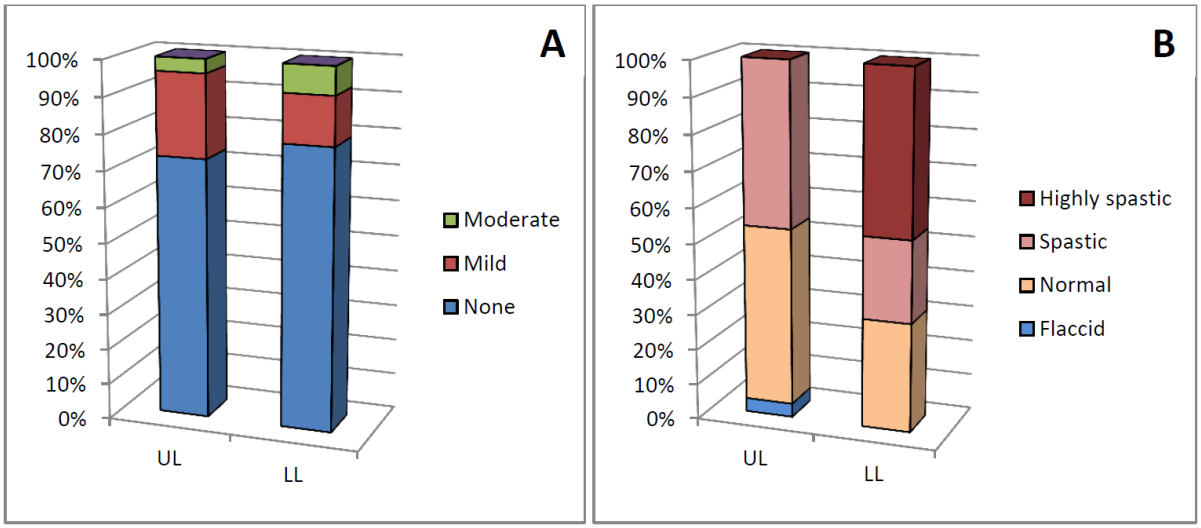

Figure 80: (A) Muscle atrophy and (B) spasticity as recorded in the SNE for ARSCACS patients

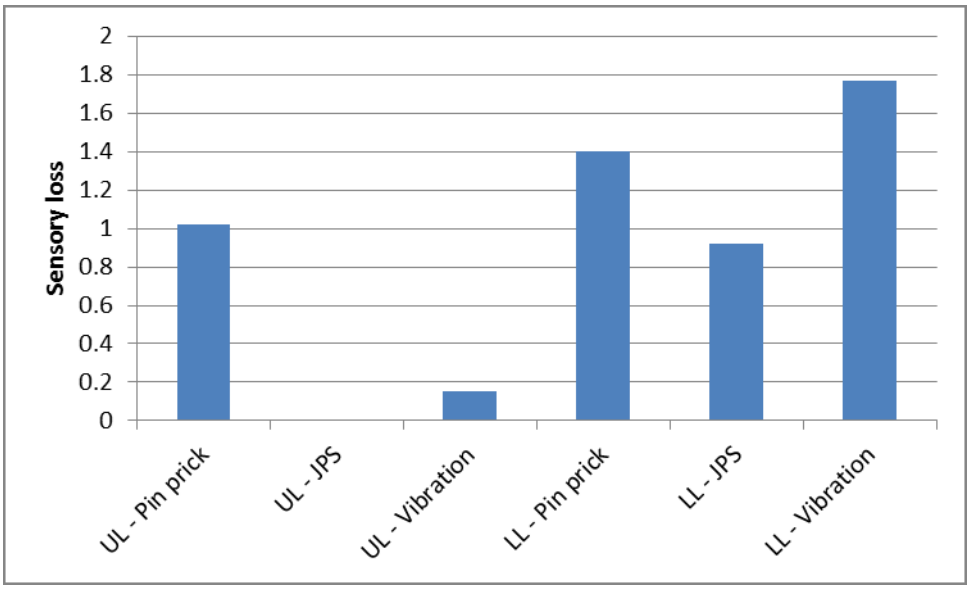

Figure 81: Extent of sensory loss as recorded in SNE for ARSACS patients.
For an explanation of the scale, see text.
UL=upper limb; LL=lower limb; JPS=joint position sense

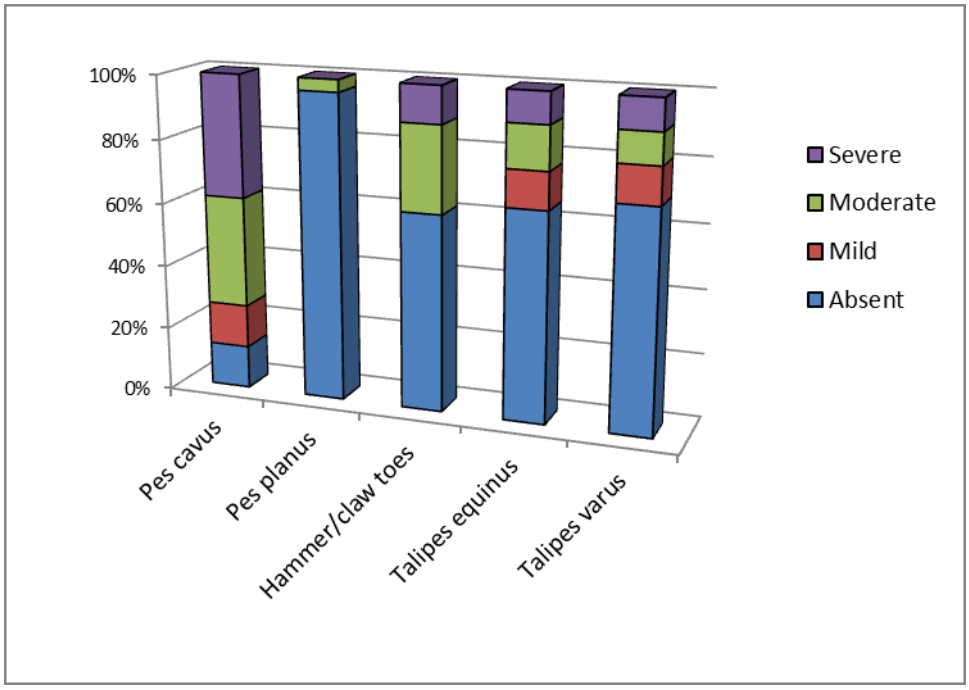

Figure 82: Skeletal foot abnormalities from SNE for ARSACS patients

Table 53 brings together all the clinical features and rating scales of the 26 patients in the study.

Table 53: Summary of all clinical findings for 26 ARSACS patients in natural history study

Clinical features are recorded on a semi-quantitative scale (0 absent; (+) equivocal; + mild; ++ moderate; +++ severe; ++++ very severe) based on the research rating scores given at the foot of the table. Reflexes are recorded as: 0 absent; (+) present only with reinforcement; + hyporeflexic; ++ normal; +++ hyperreflexic; ++++ hyperreflexic with clonus. Binary features eg extensor plantar reflexes are recorded as: 0 absent; + present.

Patient number	Sex	Age at examination	Age at onset	Disease duration	SCFS (disability score 1-7)	Age at intermittent support for walking	Age at permanent support for walking	Age at wheelchair-bound	Total ADL (0-36)	Total SARA (0-40)	INAS score (0-16)	SCAFI 8mTW (s)	SCAFI 9HPB-D (s)	SCAFI 9HPB-ND (s)	SCAFI PATA (words/10s)	Dysarthria	Dysphagia	Bladder involvement	Bowel involvement	Nystagmus	Spasticity - UL	Spasticity - LL	Ataxia - UL	Ataxia - LL	Weakness - UL proximal	Weakness - UL distal	Weakness - LL proximal	Weakness - LL distal	Biceps reflex (++ normal)	Patellar reflex (++ normal)	Ankle reflex (++ normal)	Extensor plantar reflex	Atrophy - UL distal	Atrophy - LL distal	Sensory loss - UL	Sensory loss - LL	Skeletal foot abnormalities	Epilepsy
1	M	33	0	33	5	20	26	n/a	10	21	8	n/a	35	46.5	19	+++	+	0	0	+	+	+++	+	+	0	0	0	+	++	+++	0	+	0	+	+	+	+	0
2	M	23	5	18	2	n/a	n/a	n/a	3	3.5	2	n/a	38.5	43.5	16	0	0	+	0	+	0	+	(+)	0	0	0	0	0	++	++	++	0	0	0	0	0	+	0
3	F	33	1	32	5	1	24	n/a	9	16	5	18.5	35	35.5	12	0	0	0	0	+	+	++	+	++	0	0	+	+	++	++	++	+	+	0	+	+	++	0
4	F	60	48	12	5	55	55	n/a	18	13	6	18.5	26	27.5	15	0	++	+++	0	+	0	+	+	+	0	0	(+)	0	+++	+++	0	+	0	0	+	+++	0	0
5	F	62	51	11	4	61	61	n/a	8	11	4	n/a	31.5	33.5	18	0	+	+++	0	+	+	+	+	+	0	0	0	0	++	++	++	+	0	0	+	++	0	0
6	F	61	43	18	5	51	51	n/a	13	21	8	n/a	61	54	22.5	+	+	+	0	+	0	++	++	++++	0	0	++	+	++	+++	++	+	+	0	+	0	+++	+
7	M	47	35	12	4	47	51	n/a	10	13	4	13	32.5	45	20	0	0	0	0	+	+	0	+	+	0	0	0	0	++	+++	++	+	0	0	0	+	(+)	0
8	M	60	46	14	4	55	55	n/a	6	14.5	5	n/a	60.5	75	19.5	0	0	0	0	+	+	0	+	+	0	0	+	(+)	++	++	0	+	0	0	+	+	+	+
9	M	40	2	38	6	NR	20	20	17	25.5	8	n/a	50	75.5	25	0	0	+	+	+	+	++	++	++++	0	(+)	++++	++++	0	0	0	+	0	+	0	++	++	+
10	M	45	10	35	6	10	35	43	21	26	6	9	30	34.5	11	+	++	+++	+	+	+	++	+	++++	0	+	++	++++	0	0	0	0	+	0	0	++	++	0
11	F	39	13	26	4	23	29	n/a	5	5	6	10	56	44.5	24.5	0	+	0	0	+	+	+	(+)	0	0	(+)	0	(+)	++	+++	0	+	0	0	0	+	+	0
12	M	21	2	19	3	n/a	n/a	n/a	3	9	3	11	53	62	14.5	+	0	0	0	+	0	++	+	0	0	0	0	0	++	+++	++	+	0	0	0	0	++	0
13	F	50	32	18	3	45	48	n/a	6	8.5	4	11	41	53	14	0	0	0	NR	+	0	+	0	(+)	0	0	(+)	+	0	++	++	0	0	0	0	++	+	+
14	F	32	5	27	3	25	n/a	n/a	5	9	6	13	26	28.5	24.5	++	0	0	0	+	0	+	+	0	0	0	0	0	0	+++	0	+	0	0	0	0	++	0
15	F	46	3	43	4	40	42	n/a	14	5.5	6	11.5	46	57	17.5	0	0	+++	0	+	0	0	(+)	+	0	(+)	(+)	++	0	+++	0	+	0	0	0	+	+++	+	0
16	M	51	5	46	4	49	49	n/a	14	14.5	6	n/a	36	38	17	+	+	++	0	+	+	+	+	++	0	(+)	+	+++	0	0	0	+	0	0	0	+	+++	+	0
17	M	31	1	30	5	17	25	n/a	10	16.5	5	n/a	61.5	72	19	0	0	0	0	+	0	++	(+)	(+)	0	0	(+)	++	0	++	0	+	0	0	0	+	++	0
18	M	47	13	34	5	23	42	n/a	13	21.5	8	n/a	51	99	11.5	++	0	+	0	0	0	0	+	++	0	+	(+)	+++	++	+++	0	+	0	++	0	+	+	0
19	M	50	1	49	6	30	30	40	22	28.5	6	n/a	54	177.5	16	+++	+++	++	0	+	+++	+++	++	+++	0	(+)	0	++	++	+++	++	+	0	0	0	+	+	0
20	M	46	19	27	5	21	36	n/a	15	24.5	7	n/a	n/a	n/a	12.5	+	+	+	0	+	0	+++	++	++++	0	+	+++	++++	0	0	0	+	++	++	0	+	++	0
21	F	49	3	46	6	25	39	47	19	26	8	n/a	n/a	n/a	13	+	++	+++	0	+	+	++	+	++++	(+)	++	++++	++++	++	+++	0	+	+	+	++	+++	++	0
22	M	69	34	35	6	38	38	67	26	25	9	7	47	51	13.5	0	0	++++	0	+	+	++	0	++++	(+)	++	+++	++++	++	++	0	+	+	0	0	+	+	0
23	M	24	1	23	3	21	n/a	n/a	14	11	6	n/a	44.5	49	27	++	++	0	0	+	+	+++	+	+	0	0	0	0	++	+++	0	+	+	+	0	0	++	0
24	F	57	1	56	5	47	51	n/a	12	20	6	n/a	53.5	39.5	15	+	++	+	0	+	+	+++	+	++++	0	+	(+)	+++	0	0	0	+	+	0	+	+++	+	0
25	F	31	1	30	5	26	29	n/a	17	22	6	6.5	32	31.5	29	+	++	++	0	+	+	0	+	++++	0	(+)	+	++	0	0	0	+	0	0	0	+	+	+
26	M	22	14	8	2	n/a	n/a	n/a	6	4	1	n/a	n/a	n/a	n/a	+	+	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	+	(+)	0

Research score on which clinical score above based:

SARA speech score	ADL swallow score	ADL bladder score	SNE Bowel score	INAS hoirzontal GEN score	INAS UL spasticity score	INAS LL spasticity score	SARA Nose-finger score	SARA Heel shin score	SNE UL proximal power score	SNE UL distal power score	SNE LL proximal power score	SNE LL distal power score	INAS biceps score	INAS patellar score	INAS achilles score	INAS plntar score	INAS UL distal atrophy score	INAS LL distal atrophy score	SNE UL all sensory modalities score	SNE LL all sensory modalities score	SNE all foot abnormality scores	

5.3.11 Neurophysiology

Many of the patients in the study had had nerve conduction studies as part of their diagnostic work-up. The results of 13 of these, all undertaken in the Department of Neurophysiology of the National Hospital for Neurology & Neurosurgery, were available. These were not collected prospectively as part of the study, and so in several instances had taken place several years before the patients were seen in the study, indeed often before they had a diagnosis. They were not collected in a systematic fashion or under identical conditions or following a set protocol, and were undertaken by a variety of operators. In several instances comparable results were not available. Table 54 summarizes some of the principal findings which broadly allow comparison between patients. Examples are shown of upper and lower limb motor and sensory nerve findings with compound action potentials and conduction velocities. The overall impression of the neurophysiologist at the time is quoted.

All patients had measurable compound motor action potentials (CMAP) in the median nerve at the wrist with a mean CMAP of 7.3±2.5mV (range 1.8-12.2mV; lower normal limit 4.1mV) and a mean motor conduction velocity (MCV) of 37.8±14.2m/s (range 19-58m/s; lower normal limit 49m/s). Sensory action potentials (SAP) were absent in the median nerve at the wrist in seven (58.3%) of the twelve patients in whom it was measured. In the remaining five patients, the mean SAP was 4.0±2.5μV (range 1-8μV; lower normal limit 10μV) with a mean sensory conduction velocity (SCV) of 43.8±5.5m/s (range 38-51m/s). Seven CMAP results were available for the common peroneal nerve at the ankle giving a mean CMAP of 0.4±0.5mV (range 0.1-1.4mV; lower normal limit 1.3mV). In one case the CMAP is recorded as unobtainable at the ankle. In the remaining 4 cases, the CMAP was measured at the fibular neck with mean CMAPs of 2.3±0.7mV (range 1.5-3.1mV; lower normal limit 1.7mV). This may have happened because the CMAPs were unobtainable at the ankle. The mean MCV for all 11 measurable cases in the common peroneal nerve was 33.9±7.6m/s (range 27-53m/s; lower normal limit 38m/s). SAPs were only measurable in three (25%) of the twelve patients in whom they were assessed. In these cases, the mean SAP was 4.0±1.0μV (range 3-5μV; lower normal limit 4μV) with a mean SCV of 35.3±6.8m/s (range 30-43m/s). Normal limits are quoted from (Buschbacher & Prahlow 2006).

In addition, patient 18 was previously noted to show evidence of chronic partial denervation with absent sensory and motor responses consistent with a generalized axonal neuropathy (Pyle et al. 2013). Patient 20 was described as having a large-fibre sensorimotor axonal-demyelinating neuropathy and patient 21 a mixed demyelinating axonal neuropathy (Pyle et al. 2012). Patient 23 was recorded as having a demyelinating sensorimotor neuropathy (Terracciano et al. 2010). The formal results of these investigations were not available for analysis within the current study.

Thus, in all cases there was evidence of a mixed sensory and motor neuropathy. MCVs were reduced in both upper and lower limbs in nearly all cases. CMAPs were less affected and were generally well preserved in the upper limbs. SAPs were typically absent or markedly reduced in both upper and - to a greater extent - lower limbs, with reduced conduction velocities where measurable. Overall, the neurophysiologist felt the diagnosis was consistent with a demyelinating picture in 8 (61.5%) cases, with an axonal picture in 2 (15.4%) cases and a mixed picture in 3 (23.1%) cases.

Table 54: Neurophysiological findings for ARSACS patients

Patient	Family Group	Family tree	Mutation 1	Mutation 2	Age at onset	Sensory loss	Weakness	Reflexes	Age at neurophys exam	Upper Limb				Lower Limb				Neurophysiological Impression
										Motor		Sensory		Motor		Sensory		
										Median Nerve		Median Nerve		Common Peroneal Nerve		Sural Nerve		
										CMAP wrist (mV)	MCV wrist-elbow (m/s)	SAP F2-wrist (µV)	SCV F2-wrist (m/s)	CMAP ankle (mV)	MCV fibular neck-ankle (m/s)	SAP calf-ankle (µV)	SCV calf-ankle (m/s)	
1	A	III-4	c1144G>T	c11352_11353dup	1	(+)	(+)	N/-/++	30	7	43	absent	-	3.1*	53	absent	-	DSMN
2	B	III-1	c7255_7259del	c9956_9957delAA	5	-	-	N	19	8.7	41	absent	-	NR	NR	absent	-	DSMN
3	C	II-2	c5820_5821del	c7162_7163del	1	++	+	N	21	9.7	40	1	38	0.2	31	absent	-	DSMN
5	D	III-3	c8339T>G; c12416T>C	c11675C>G	51	++	-	N/-	58	8.1	44	3	48	0.7	28	5	30	ASMN
6	D	III-5	c8339T>G; c12416T>C	c11675C>G	43	+	+	N/++	52	8.5	55	8	51	0.3	41	4	43	ASMN
7	D	III-13	c8339T>G; c12416T>C	c11675C>G	35	+	-	N/++	54	7.6	44	4	42	1.4	30	absent	-	DASMN
8	D	III-7	c8339T>G; c12416T>C	c11675C>G	46	+	+	N/-	58	4.8	40	4	40	0.1	31	3	33	DSMN
9	E	III-1	c5151dupA	c5948C>T; c6392delT	2	++	+++	-	33	7	38	absent	-	1.5*	27	absent	-	DSMN
10	F	III-1	c9404T>C	c11265_11266delAT	10	++	+++	-	32	1.8	34	absent	-	1.9*	29	absent	-	DASMN
11	F	III-2	c9404T>C	c11265_11266delAT	13	++	+	N/++	24	4.9	40	absent	-	0.3	33	absent	-	DASMN
13	H	III-2	c4226_4229del	c9404T>C	32	++	+	N/-	48	7.9	41	NR	NR	0.1	38	NR	NR	DSMN
14	I	III-3	c3149C>A	c4744G>T	5	-	-	N/-/++	33	6.9	39	absent	-	2.5*	32	absent	-	DSMN
15	J	II-1	c9404T>C	c12028C>T	23	+++	+	N/-	30	12.2	36	absent	-	absent	-	absent	-	DSMN
								Mean:	37.8	7.3	41.2	1.7	18.3	1.0	31.1	1.0	8.8	
								SD:	14.2	2.5	5.1	2.6	22.8	1.1	12.2	1.9	16.2	
								Min:	19	1.8	34	0	0	0	0	0	0	
								Max:	58	12.2	55	8	51	3.1	53	5	43	
								Count:	13	13	13	12	12	12	12	12	12	

*recorded at fibular neck, not ankle
NR=not recorded
DSMN=Demyelinating sensorimotor neuropathy
ASMN=Axonal sensorimotor neuropathy
DASMN=Demyelinating axonal sensorimotor neuropathy
CMAP=Compound motor action potential
MCV=Motor conduction velocity
SAP=Sensory action potential
SCV=Sensory condution velocity

5.3.12 Neuropathology

Five patients underwent quadriceps muscle biopsy (patients 6, 9, 11, 13 & 17) and three sural nerve biopsy (patients 9, 11 & 13). This was usually during the diagnostic process and often long before inclusion in the current study. The results were collated from the previous notes and not collected prospectively as part of this study. The results are shown in Table 55. The muscle biopsies were generally uninformative as to the diagnosis but did not show evidence of mitochondrial cytopathy except in the case of patient 13. Most consistently they showed an excess of type I muscle fibres suggestive of denervation and reinervation. The nerve biopsies generally showed loss of large myelinated axons and little evidence of demyelination.

Patient 9 also underwent skin biopsy looking for Lafora body disease. This showed globular, discrete periodic acid-Schiff-positive inclusions within the apocrine glands resembling Lafora bodies but Lugol's iodine reaction was negative and ultrastructurally they contained filamentous aggregates, features not typical of Lafora bodies. There was prominent lipofuscin present in basal myoepithelial cells. This observation has previously been reported (Stevens *et al.* 2013) who raised the question of whether ARSACS might represent a lysosomal storage disorder or a condition of abnormal protein aggregation.

In addition, a muscle biopsy on patient 18 has previously been reported as demonstrating angular fibres, small group atrophy, fibre type grouping and type II fibre predominance consistent with neurogenic changes (Pyle et al. 2013). Patient 21 showed type I fibre clustering (Pyle et al. 2012). The formal results of these investigations were not available for the current study.

Table 55: Muscle & nerve biopsy results for ARSACS patients in study

Patient	Age at onset	Age at nerve biopsy	Nerve biopsy result	Age at muscle biopsy	Muscle biopsy result
6	43	-	Not done	54	Occasional polygonal atrophic fibres. Small number of nuclear bag fibres. No increase in connective tissue or internal nuclei. No regeneration, necrosis or inflammation. No ragged red or cytochrome oxidase negative fibres. Lipid and glycogen content normal. Acid phosphatase, phosphorylase and adenylate deaminase activities normal. Type I fibre predominance.
9	2	30	Some myelinated axons are atrophic but there is no evidence of degeneration, regeneration, demyelination or onion bulb formation. Many myelinated and unmyelinated axons contain more electron dense bodies than is normal, and contain varying amounts of neurofilament fibrils. There are fewer large myelinated axons than normal.	29	No significant atrophy or increase in connective tissue. No regeneration, necrosis or inflammation seen. No ragged red or cytochrome oxidase negative fibre. Lipid content slightly increased. Glycogen normal. Acid phosphatase, phosphorylase & adenylate deaminase activities normal. Fibre typing shows disturbance of the normal pattern with only scanty type IIa fibres present. The presence of a slight increase in lipid content raises the possibility of a metabolic myopathy. No evidence of mitochondrial myopathy.
11	13	32	Reduced density of large myelinated fibres and large axons. Significant endoneurial oedema. Negative for amyloid. A mild neuropathy particularly affecting large myelinated axons with a small demyelinating component. The lack of inflammatory cell activity and the homogenous nature of the pathology favours a genetic cause rather than an acquired neuropathy.	32	No increase in connective tissue or internal nuclei. No regeneration, necrosis or inflammation. No ragged red or cytochrome oxidase negative fibres. Slight increase in lipid content. Glycogen content normal. Acid phosphatase adenylate deaminase & phosphorylase activities normal Excess of type I fibres. Degree of type IIb fibre atrophy. No evidence of denervation.
13	32	NK	Moderately severe axonal neuropathy with selective loss of large fibres & some regeneration. No evidence of demyelination.	NK	Small numbers of cytochrome oxidase negative fibres suggestive of mitochondrial myopathy. Some denervation and reinervation. EM studies showed collections of mitochondria with free glycogen but no para-crystalline inclusions. Respiratory enzyme studies consistent with a degree of heteroplasmy and indicated reduced complex 4 activity.
17	1	-	Not done	25	Scattered & clustered polygonal atrophic fibres. No increase in connective tissue or internal nuclei. No regeneration, necrosis or inflammation. No ragged red or cytochrome oxidase negative fibres. Fibres have normal internal architecture. Lipid & glycogen content normal. Acid phosphatase and adenylate deaminase activities are normal. Fibre typing reveals small groups of all 3 fibre types. The atrophic fibres are predominantly type 2b fibres and likely to represent a degree of previous denervation with reinervation. No features of mitochondrial disease.

5.3.13 Neuroimaging

Sixteen patients underwent MR imaging of the neuraxis as part of their diagnostic work-up and subsequent clinical monitoring. All 16 had imaging of the brain and a further 12, imaging of the spinal cord. The results were obtained by reviewing the neuroradiological reports of previous imaging which was not collected prospectively as part of this study. The results are summarized in Table 56. Thirteen patients (81.3%)

were noted to have cerebellar volume loss, which was predominantly in the superior vermis, but to a lesser extent in the cerebellar hemispheres or cerebellar peduncles. Eight patients (66.7%) showed thinning of the spinal cord. Four patients (25%) were recorded as having pontine hypodensities. Corpus callosal thinning was observed in three cases (18.8%) and generalized cerebral volume loss in a further three. More focal parietal volume loss was seen in 2 cases (12.5%).

In addition, MR imaging on patient 18 was previously reported as showing marked cerebellar vermian atrophy and reduced spinal cord diameter (Pyle et al. 2013). The MRI of patient 20 showed cerebellar atrophy and that of patient 21, generalized atrophy most prominently affecting the cerebellum (Pyle et al. 2012). Patient 23 was reported to have a severely atrophied upper cerebellar vermis as well as pontine tigroid hypointensities (Terracciano et al. 2010).

Table 56: Neuroimaging features of ARSACS patients in natural history study

Patient	Age at onset	Age at MRI	Cerebellar volume loss	Spinal cord thinning	Pontine tygroid hypodensitiies	Nature of cerebellar volume loss	Other features
1	1	24	+	-	-	Superior vermis	-
2	5	19	++	$+^{(T)}$	-	Superior vermis, hemispheres & superior peduncles	-
3	1	25	-	$+^{(T)}$	+	n/a	Signal change ventrolateral thalami
4	48	59	+	-	+	Superior vermis	Posterior corpus callosal volume loss
5	51	58	-	ND	-	n/a	-
6	43	61	-	ND	-	n/a	-
7	35	53	+	ND	-	Superior vermis & hemispheres	Mild generalized cerebral atrophy
8	46	57	+	-	-	Superior cerebellum	-
9	2	37	++	$+^{(D)}$	+	Superior vermis & peduncles	Mild generalized cerebral atrophy, especially in parietal region. Corpus callosal thinning
10	10	43	+	$(+)^{(D)}$	-	Hemispheres	Incidental cisterna magna
11	13	31	(+)	ND	-	NR	-
13	32	48	+	$+^{(C)}$	+	Hemispheres	Mature right cerebellar infarct. Left mesial temporal sclerosis. Parietal volume loss.
14	5	32	++	$+^{(C)}$	-	NR	-
15	23	45	+	$++^{(C)}$	-		Generalized cerebral atrophy. Abnormal signal posterior internal capsule.
17	1	29	+	$+^{(C)}$	-	Vermis	Corpus callosal thinning
25	1	22	(+)	-	-	Vermis	-

NR=not recorded; n/a=not applicable
C=Cervical; T=thoracic; D=diffuse

5.4 Discussion

The analysis described herein represents the largest single centre prospective natural history case series of ARSACS patients described outside Charlevoix & Saguenay, and the first large systematic clinical and genetic study of ARSACS in the UK. It describes the clinical features of patients bearing nine completely novel pathogenic *SACS* gene mutations, as well as those of a further five mutations which have previously only been briefly described from a genetic point of view (Vermeer 2012). It applies a series of research-based rating scales which have not hitherto been evaluated in ARSACS patients and whose knowledge is vital in the planning of future drug trials. Since the data herein were collected alongside and using an analogous protocol to a large systematic prospective natural history study of FRDA, it enables direct comparison with the commonest and closest genetic condition in the differential diagnosis of ARSACS.

The 26 patients in the study bear 30 pathogenic mutations which are predominantly point mutations (deletions, duplications and substitutions causing largely premature termination of the protein either directly or by frameshift effect) but include two large deletions of 0.7 & 1.5Mb, both of which have previously been published (Terracciano *et al.* 2010, Pyle *et al.* 2013). Sixteen of the mutations have previously been described. These do not include either of the common mutations thought to represent founder mutations in the Québecois population (c.8844delT & c.7504C>T), and only one of the rarer mutations subsequently identified amongst this population (c.4744G>T) (Thiffault et al. 2013, Engert *et al.* 2000). This is significant as the Québecois cases were the first to be described and remain the most numerous single population studied; their clinical features therefore dominate the world literature. When cases were identified outside Québec, they were often described as atypical (Baets et al. 2010), most prominently having significantly later onset. It may be that these features represent part of the normal clinical spectrum of ARSACS in the genetically more heterogeneous worldwide population.

In keeping with previous studies which describe a large number of private mutations, the current study has identified nine completely novel mutations and a further five hitherto not described in the peer-reviewed literature. These consist of eight nonsense mutations which are considered obligately pathogenic, and six missense mutations for which *in silico* analysis implies pathogenicity. Although several of the mutations described are shared by multiple family members as would be expected, only two mutations are found in individuals not known to be related: the nonsense mutation c.9956_9957delAA is seen in patients 2 & 17, whilst the missense mutation c.9404T>C appears to be more common in the UK as it is present in five individuals from three families (patients 10,11,13,15 & 16 from families F, H & J). The current study is important as, for the first time, it provides an overview of the clinical and genetic spectrum of ARSACS in the UK. However, the current evidence suggests that as further cases are identified, they may well have further unique mutations and therefore potentially different clinical features.

There has been no consistent gender preponderance reported previously in ARSACS, with the male:female ratio varying in previous published cohorts from 1:0.4 (El Euch-Fayache *et al.* 2003) to 1:1.6 (Pilliod et al. 2015); the ratio in the present study of 1:0.7 is therefore not inconsistent with these. All the participants in this study were Caucasian in origin. This is in keeping with previous studies, as although cases from Japan and the Maghreb have been reported, no Afro-Caribbean or South Asian cases have been identified.

In common with previous studies, nearly half the patients had disease onset before the age of 5. However, the mean age at onset (15.0±17.4), age at examination (43.4±13.7) and disease duration (28.5±12.9) of patients in the study are greater than in most other published cohorts. This may be because there are no children in the study, but is also partly driven by a single family (family D) containing five affected members with unusually late onset of between 35 and 51; the latter is one of the latest onset cases yet described. It seems likely this is a genetic effect. This family all share two missense mutations in *cis* (c.8339T>G & c.12416T>C) which are predicted to be pathogenic, and a nonsense mutation (c.11675C>G) whose effect is to truncate the protein toward its carboxyl-terminus (at amino acid 3892 out of

4579). These may therefore represent less severe mutations. Genotype-phenotype correlations are difficult in a small cohort containing a large number of mutations where each patient has at least two contributory pathogenic mutations. The siblings within families F & J also seem to have similar ages at onset, but there is marked variation within families L, M & P.

In common with FRDA, the predominant symptoms at onset were gait problems and falls, although unlike FRDA, the majority of patients maintained the ability to mobilize by the time they were seen as part of the study. The ADL assessment shows that walking remains the most affected function studied causing frequent falls. Speech, swallowing and activities involving fine manual dexterity such as dressing, washing and use of cutlery, are comparatively less affected. The SARA results mirror this functional finding with gait and stance most affected, and lower limb measures of ataxia more severely affected. The INAS shows that both distal and proximal lower limb weakness are common in ARSACS. There is often mild distal upper limb weakness but rarely significant proximal weakness. Spasticity is common and more severe in the lower limbs than the upper with the majority of patients having both upper and lower limb spasticity. Muscle cramps are common and rated as severe in nearly are half of all patients. There is commonly a mixed pattern of reflexes reflecting the neuropathy and pyramidal features seen in this condition. This is most prominently displayed by the patellar reflex for which half of the patients are hyperreflexic, a quarter areflexic and a quarter normal. For the biceps and ankle jerks, patients are far less commonly hyperreflexic and far more frequently areflexic. Extensor plantar reflexes are seen in more than 80% of cases. Regarding the eye movements, broken smooth pursuits and horizontal gaze-evoked nystagmus are the most common feature which are almost universally found.

The SNE provides a much more detailed view of the examination findings, particularly those not studied in the SARA and INAS. This confirms preservation of muscle power in the upper limbs especially proximally where there is very rarely weakness. Lower limb power is more severely affected, particularly distally. The reflexes are recorded in a slightly different way in the SNE from the INAS but the results are broadly comparable. The supinator and triceps reflexes which are not

evaluated as part of the INAS, show similar findings to the biceps reflexes : they are rarely brisk, normal in just over half of cases and absent in 30-40% of cases. Finger jerks and Hoffman's reflexes are present in only a very small fraction of cases. Muscle atrophy is present in approximately a quarter of cases, and then usually very mild. Sensory loss is common and more prominent in the lower limbs. Lower limb vibrational sense is the most severely affected, with over half of patients recorded as severely affected at the ankle by the INAS, and most extensively impaired by the SNE. Pin prick and proprioceptive sensory loss is also common in the lower limbs. In the upper limbs, distal pin prick loss was seen much more commonly that vibrational loss; proprioceptive loss was not observed in the upper limbs. Light touch and temperature sensation were not assessed as part of this study. A range of skeletal foot abnormalities were seen : pes cavus is almost always found, with hammer or claw toes or talipes equinovarus seen in 30-40% of cases.

Nerve conduction studies consistently showed evidence of a mixed sensory and motor neuropathy with reduced conduction velocities and to a lesser extent action potentials, affecting the lower more than the upper limbs. These findings were most consistent with a demyelinating neuropathy with an axonal component. However, the few cases which had nerve biopsies generally showed loss of large myelinated axons and little evidence of demyelination. Muscle biopsy typically shows an excess of type I muscle fibres suggestive of denervation and reinervation and is rarely informative, although usually excludes a mitochondrial cytopathy which may be under consideration during the diagnostic work-up.

The most common features on neuroimaging are superior vermian cerebellar atrophy and spinal cord thinning, most commonly in the cervical region. Pontine tigroid hypodensities, which are probably a more specific feature of ARSACS, were only seen in 25% of this cohort. However, the imaging results were not collected prospectively and the pons may not have been specifically scrutinized for these changes. The sensitivity and specificity of this feature warrants further investigation with a prospectively planned study and a protocol designed specifically to study this feature.

Epilepsy was seen in 5% of the Québecois patients but has only variably been reported in subsequent cohorts. It was seen at similar levels in two predominantly European studies (Vermeer *et al.* 2008, Baets *et al.* 2010) and up to 9% in a similar, more recent but larger study (Pilliod *et al.* 2015). In the present study, epilepsy was reported in 19% of cases, making this the highest prevalence reported to date in any natural history series. Of note, one patient (patient 13), was has mesial temporal sclerosis on MR imaging which may independently explain her diagnosis of epilepsy. Whether epilepsy is causally linked with ARSACS is still unclear, but it may well have been overlooked in previous studies and should be more systematically studied in the future. The higher prevalence in the present study may represent particular genetic features of the UK cases. Interestingly, 19% of patients also complained of headaches.

Urological complications such as urinary frequency, incontinence and retention were commonly seen, affecting over a quarter of cases. These features are frequently overlooked and are often treatable. Approximately 15% of patients complained of depression. Other general medical conditions including asthma, hypertension, hypercholesterolaemia and common eye conditions were present at greater than 10% frequency, likely reflecting there prevalence in the general population. Mitral valve prolapse was reported in 57% of the original cohort of ARSACS patients (Bouchard *et al.* 1978) but was not observed in subsequent case series. The current series has also not found evidence of valvular cardiac problems.

Thus, this study confirms previous descriptions of ARSACS as causing early onset gait disturbance and falls, followed by spasticity, ataxia and distal sensory loss which predominantly affect the lower limbs. Muscle atrophy is less common. The deep tendon reflexes show a mixed pattern with areflexia commoner than hyperreflexia, but the two frequently coexisting in the same patient. The plantar reflexes are typically extensor. Skeletal foot abnormalities are very common with pes cavus most frequently present. A predominantly demyelinating sensorimotor neuropathy is found on neurophysiological testing. Taken together, these findings confirm the coexistence of cerebellar, pyramidal and neuropathic features which have been more systematically evaluated than previously. This study also confirms

the common existence of late-onset forms of ARSACS which were not seen amongst the originally described populations in Charlevoix and Saguenay. However, it does raise the suspicion of an association with epilepsy which was described in the original population. This may have been underestimated in subsequent studies and merits further investigation.

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Chapter 6 : Ocular Coherence Tomography in Diagnosing Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay

6.1 Introduction

Within the earliest clinical descriptions of ARSACS, Bouchard observed 'striking and markedly increased visibility of the retinal nerve fibers, mainly in the papillomacular bundle area' akin to the changes seen in the early stages of Leber's hereditary optic neuropathy (LHON) (Bouchard *et al.* 1978). The fundoscopic changes appear as prominent streaks emanating in all directions from the optic disc, most striking in the papillomacular bundle and nasal to the disc where such striations are rarely visible in healthy people. The retinal vessels, which normally lie on the surface of the nerve fibre layer, may be buried within this thickened layer obscuring their normally crisp margins (see Figure 83 A & B). These retinal striations are often described in the literature as composed of myelinated (Bouchard 1991) or hypermyelinated (Takiyama 2006, Prodi *et al.* 2013) retinal fibres, although their exact nature remains unknown as no histopathological studies of the eyes in ARSACS have yet been published.

However, these retinal changes are not consistently observed on fundoscopy, particularly in non-Québecois cases of ARSACS, such as those from Japan, Turkey, Tunisia, Italy, the Netherlands and Spain (Takiyama 2006, Gücüyener *et al.* 2001, Mrissa *et al.* 2000, Grieco *et al.* 2004, Garcia-Martin *et al.* 2013, Vermeer *et al.* 2008), supporting the idea of increasing phenotypic variability associated with the increasing genetic diversity of cases identified outside Québec. A more sensitive method of detecting them appears to be ocular coherence tomography (OCT). In ARSACS, OCT shows thickening of the retinal nerve fibre layer (RNFL) in all sectors around the disc, with average peripapillary thicknesses of between 119 and 220μm (Vingolo *et al.* 2011, Pablo *et al.* 2011, Desserre *et al.* 2011, Garcia-Martin et al. 2013, Stevens *et al.* 2013, Nethisinghe *et al.* 2011, Gazulla *et al.* 2012). In the macula, RNFL thickening extends over the fovea and can obscure the foveal pit (see Figure 83Figure C) (Nethisinghe *et*

al. 2011). Only one series has reported absence of RNFL thickening on OCT, in a single case with RNFL thicknesses of 86 and 111μm (Yu-Wai-Man *et al.* 2014). It has also been reported that unaffected heterozygous carriers of two different *SACS* mutations in a single family showed partial RNFL thickening on OCT of up to 119μm thickness (Nethisinghe *et al.* 2011).

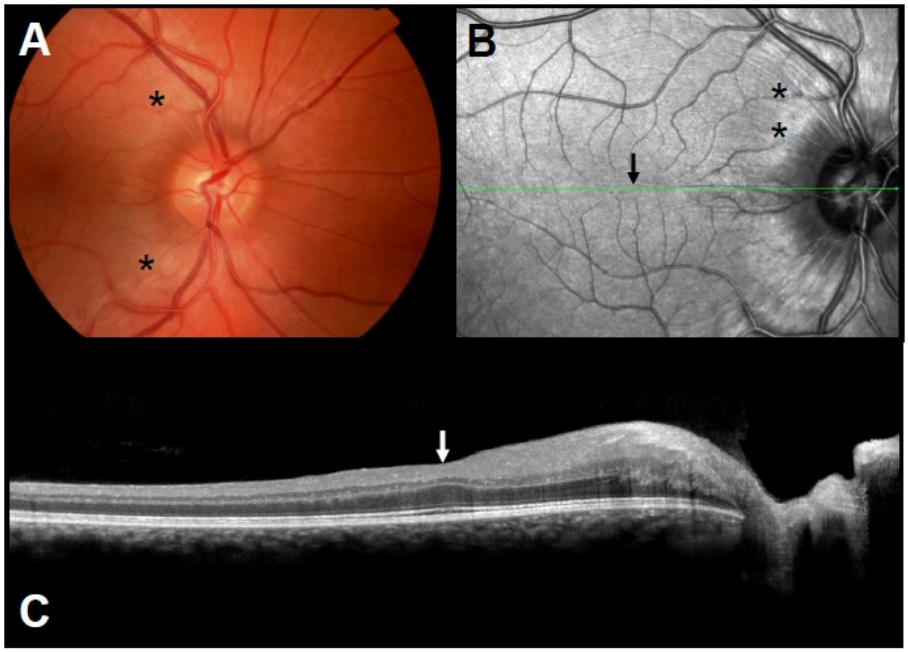


Figure 83: Retinal appearance in ARSACS (A) in colour; (B) red-free image; (C) OCT
The optic disc is normal but there is visible thickening of the RNFL in all quadrants; in some places this has obscured the normally sharp edges of the retinal vessels (asterisks). OCT scan along green line in image (B) shows thickening of both ganglion cell and nerve fibre layers extending across the fovea (arrowed).
Image courtesy of Dr Fion Bremner, NHNN.

OCT provides micron-scale, cross-sectional images (tomographs) of biological tissue to a depth of a few millimetres acquired by non-invasive means without direct contact (see Figure 84). The resulting images of the retina are of such quality that they have been described as 'optical biopsies' comparable to non-invasive histological sections (Anderson *et al.* 2011). The technique is analogous to B-mode ultrasound or radar but uses a focused beam of light, typically 800-1400nm in the near infra-red region. This is split with half scanned across the tissue in question and half directed to a moving reference mirror. The two reflected beams are recombined in a Michelson-type interferometer which detects interference between the two light signals and so time-of-flight delay caused by reflection from different histological layers. As the light beam

is scanned across the tissue, multiple individual A-scans showing the interference pattern are obtained, which are combined to form a B-scan showing a 2-dimensional cross-sectional image of the tissue. This is usually displayed as either a grey-scale or false-colour image (Costa *et al.* 2006, Anderson et al. 2011, Jindahra *et al.* 2010).

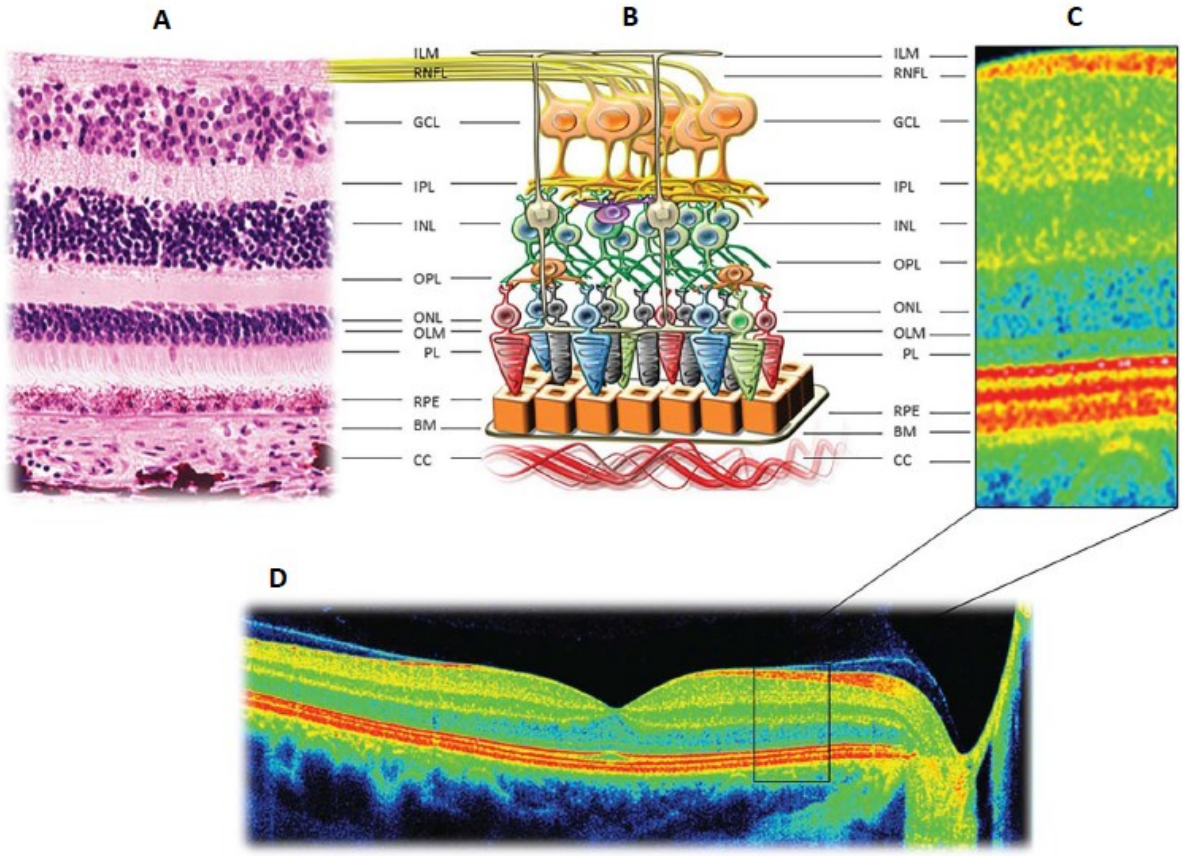


Figure 84: OCT images of the human retina and corresponding histopathological layers

(A) Haematoxylin & eosin stain of human retina; (B) schematic representation of human retina; (C) colourized cross section of human retina by SD-OCT taken from (D). Image from (Galetta *et al.* 2015)

ILM=inner nuclear layer; RNFL=retinal nerve fibre layer; GCL=ganglion cell layer; IPL=inner plexiform layer; INL=inner nuclear layer; OPL=outer plexiform layer; ONL=outer nuclear layer; OLM=outer limiting membrane; PL=photoreceptor layer; RPE=retinal pigment epithelium; BM=Bruch membrane; CC=choriocapillaris layer.

Two OCT methodologies are available. The first to be developed was time-domain OCT (TD-OCT). This uses a linear or circular scan, often positioned around the optic nerve head (peripapillary view) or macula (perifoveal view), to produce a single 2-dimensional image. Acquisition times can be up to several seconds which can introduce artefact due to eye movements, and the scan is usually positioned manually

by the examiner, potentially leading to variation between patients. More recently, spectral-domain OCT (SD-OCT) has been introduced in which the reference mirror is kept stationary, and the reflected light is analyzed according to variation in wavelength rather than time. Acquisition times are much shorter and spatial resolution much greater. The larger amount of data obtained is analyzed by Fourier transform allowing the production of 3-dimensional images known as OCT fundus images. The decreased acquisition time reduces movement artefact and scanning of the whole fundus allows automated fixation improving variation between patients and allowing comparable sequential measurements in the same patient (Jindahra et al. 2010, Anderson et al. 2011, van Velthoven *et al.* 2007).

OCT has been used extensively in studying retinal disease and glaucoma, including the effects of drugs on the retina. Thinning of the RNFL has been demonstrated after optic neuritis, neuromyelitis optica and non-arteritic anterior ischaemic optic neuropathy (NAION). In the neurological sphere, OCT has been mostly extensively studied in multiple sclerosis with correlations found between RNFL thinning and various measures of disease progression. RNFL thinning has also been described in Alzheimer's disease and Parkinson's disease although with less consistent correlations with clinical parameters or other markers of neurodegeneration (Jindahra et al. 2010). Amongst the ataxic conditions, peripapillary RNFL thinning has been demonstrated in spinocerebellar ataxia (SCA) types 2, 3 and 7, and perifoveal thinning in SCA1, 3, 6 and the cerebellar subtype of multiple system atrophy (MSA-C) (Pula *et al.* 2011, Manrique *et al.* 2009). In Friedreich's ataxia (FRDA), peripapillary RNFL thicknesses have been shown to be reduced with correlations to markers of clinical severity and the underlying genetic process (Fortuna *et al.* 2009, Noval *et al.* 2012, Seyer *et al.* 2013). Thus, thinning of the peripapillary RNFL appears to be a common feature of neurodegenerative disease, whether or not visual loss is a major feature.

By contrast, thickening of the RNFL is rarely seen in the context of chronic progressive neurodegenerative disease. Most commonly it is seen as a result of acute optic disc oedema associated with the acute phases of inflammatory optic neuropathy, NAION, retinal vein occlusion or other causes of papilloedema (Karam & Hedges 2005, Savini *et al.* 2006). This commonly changes over time. For example, in the acute phase of LHON,

the RNFL is acutely significantly thickened but later in the atrophic phase of this condition (more than 6 months after onset), the RNFL becomes significantly thinned (Barboni *et al.* 2005). OCT studies in patients with retinitis pigmentosa have shown RNFL thickening in the absence of optic disc swelling (Hood *et al.* 2009); however in these cases there is gross disruption to the outer retinal layers making interpretation of these RNFL measurements unclear. RNFL thickening is therefore not normally seen in the context of chronic progressive neurodegenerative disease. ARSACS may be an exception to this rule.

It is therefore not clear whether RNFL thickening as detected by OCT is present in all cases of ARSACS, nor is it known whether similar OCT changes may be seen in patients with other non-ARSACS causes of ataxia or asymptomatic carriers of *SACS* mutations. The purpose of the present study is to measure RNFL thickness in patients with ARSACS and compare these measurements to those in a large cohort of patients referred to a tertiary hospital specialist ataxia clinic with other types of ataxia, as well as first degree relatives of known patients with ARSACS. Nearly 200 different mutations have now been described in the *SACS* gene. The large size of the gene and diversity of mutations means that diagnostic testing usually requires sequencing of the entire gene which is expensive, time-consuming and is not available in many centres. There is therefore a pressing need for a reliable surrogate test for ARSACS. Assessment of RNFL thickness by OCT may provide such a test.

Table 57: Demographic & OCT results by disease group

Disease	Number of participants	Number of eyes examined	Age [mean ± SD (range)]	M:F ratio	OCT-S [mean ± SD (range)]	OCT-N [mean ± SD (range)]	OCT-I [mean ± SD (range)]	OCT-T [mean ± SD (range)]	OCT-Av [mean ± SD (range)]
ARSACS	17	34	42.8 ± 13.4 (21-61)	9:8	172.6 ± 19.2 (145.0-200.0)	126.4 ± 18.3 (95.5-152.5)	185.3 ± 21.1 (146.5-225.5)	115.7 ± 21.6 (78.5-153.5)	150.0 ± 16.0 (119.3-174.8)
ARSACS carriers	13	26	47.2 ± 16.9 (25-70)	5:8	115.8 ± 11.2 (94.5-128.5)	78.1 ± 16.4 (54.5-96.5)	120.0 ± 13.4 (92.0-145.5)	61.3 ± 9.1 (50.5-81.5)	93.8 ± 7.5 (82.0-105.5)
FRDA	59	113	31.7 ± 11.7 (15-61)	32:27	88.5 ± 25.2 (34.0-150.5)	59.4 ± 20.2 (29.5-140.5)	91.5 ± 21.5 (39.0-134.5)	59.5 ± 11.9 (30.0-84.5)	74.7 ± 15.1 (35.1-104.2)
SCAs	53	96	55.6 ± 14.1 (24-81)	30:23	103.3 ± 20.4 (40.5-136.5)	63.1 ± 17.3 (22.0-114.0)	106.0 ± 20.1 (45.0-139.0)	63.4 ± 18.2 (34.0-130.0)	83.9 ± 13.0 (40.2-103.5)
SCA1	5	9	42.2 ± 8.5 (30-54)	3:2	105.5 ± 22.7 (76.0-132.5)	68.5 ±18.7 (41.0-83.5)	100.9 ± 26.3 (57.0-120.0)	57.6 ± 11.4 (40.0-68.0)	83.1 ± 18.5 (57.3-101.0)
SCA2	10	18	48.9 ± 11.9 (28-65)	3:7	95.0 ± 25.2 (40.5-122.5)	60.6 ± 13.7 (40.0-79.0)	104.0 ± 24.8 (45.0-135.0)	56.1 ± 12.2 (36.0-77.0)	78.8 ± 16.8 (40.2-98.0)
SCA3	9	13	52.7 ± 10.4 (35-70)	5:4	95.2 ± 18.6 (73.0-136.0)	52.8 ± 19.5 (22.0-81.5)	98.9 ± 15.5 (77.0-121.0)	77.4 ± 31.6 (41.0-130.0)	81.0 ± 7.8 (67.2-94.0)
SCA6	20	39	64.5 ± 11.3 (43-81)	12:8	108.7 ± 18.1 (74.0-136.5)	67.5 ± 17.9 (31.0-114.0)	113.0 ± 16.4 (71.5-139.0)	65.0 ± 13.7 (50.5-103.5)	88.5 ± 10.7 (65.6-103.5)
SCA7	6	12	45.8 ± 16.3 (24-64)	5:1	102.4 ± 20.4 (68.4-128.5)	63.4 ± 18.2 (40.0-81.0)	94.1 ± 19.4 (62.5-113.0)	53.4 ± 9.9 (34.0-60.5)	78.3 ± 14.6 (51.3-90.8)
SCA14	2	3	71.0 (67-75)	1:1	110.0 (106.0-114.0)	61.5 (56.0-67.0)	129.8 (124.0-136.0)	68.3 (60.0-77.0)	92.0 (90.1-94.0)
SCA28	1	2	65	1:0	131	69	99	60	89.4
Other genetic ataxias	17	32	52.1 ± 16.4 (25-77)	7:10	107.6 ± 20.1 (74.5-143.5)	64.7 ± 22.0 (34.0-108.0)	114.3 ± 21.7 (59.0-144.5)	64.1 ± 16.1 (46.0-106.5)	87.7 ± 14.1 (56.3-106.3)
AOA2	6	12	44.2 ± 17.1 (25-73)	3:3	115.3 ± 22.9 (85.5-143.5)	68.5 ± 21.7 (43.5-100.5)	123.5 ± 12.9 (110.5-144.5)	81.2 ± 14.8 (67.5-106.5)	97.0 ±8.9 (85.4-106.3)

HSP7	4	8	61.0 ± 13.6 (47-77)	3:1	116.6 ± 14.6 (103.5-129.5)	72.3 ± 18.2 (52.0-94.5)	119.8 ± 4.9 (114.0-124.5)	53.4 ± 7.5 (46.0-63.0)	90.4 ± 8.1 (85.1-102.3)
EA1	2	4	51.5 (35-68)	0:2	103.5 (90.5-116.5)	88.0 (68-108)	119.5 (100.5-138.5)	55.0 (53-57)	91.6 (79.0-104.2)
EA2	1	2	44	0:1	99.0	53.0	139.0	58.0	88.5
FXTAS	1	2	72	1:0	74.5	43.5	100.5	46.0	66.4
FGF	1	2	60	0:1	111.0	51.0	78.0	56.0	73.7
SLS	1	1	33	0:1	76.0	34.0	59.0	56.0	56.3
ACoQ$_{10}$D	1	1	64	0:1	105.0	43.0	109.0	65.0	80.0
iCA	45	83	50.3 ± 15.2 (21-82)	16:29	112.2 ± 20.7 (52.0-144.5)	72.4 ± 17.3 (39.0-115.0)	117.5 ± 22.2 (61.0-159.5)	62.9 ± 14.4 (30.0-93.5)	91.5 ± 13.4 (45.5-124.0)
All non-ARSACS (excluding carriers)	174	325	45.8 ± 17.2 (15-82)	85:89	101.0 ± 24.0 (34.0-150.5)	64.4 ± 19.3 (22.0-140.5)	104.9 ± 23.6 (39-159.5)	62.0 ± 15.1 (30.0-130.0)	83.1 ± 15.4 (35.1-124.0)

ARSACS=Autosomal recessive spastic ataxia of Charlevoix-Saguenay; FRDA=Friedreich's ataxia; SCA=Spinocerebellar ataxia; AOA=Ataxia with oculomotor apraxia
HSP=Hereditary spastic paraparesis; EA=Episodic ataxia; FXTAS=Fragile X tremor ataxia syndrome; FGF=Ataxia with fibroblast growth factor mutation
SLS=Sjögren-Larsson syndrome; ACoQ$_{10}$D=Ataxia with co-enzyme Q$_{10}$ deficiency (ADCK3 mutation)
iCA=idiopathic cerebellar ataxia
S=superior ; N=nasal ; I=inferior ; T=temporal ; Av=average over all quadrants
SD=standard deviation

6.2 Subjects and Methods

6.2.1 Study Population & Ethical Approval

Patients were recruited from the Ataxia Centre of the National Hospital for Neurology and Neurosurgery (NHNN), which receives referrals for specialist opinions from across the UK and internationally. The majority of patients live in London and the South-East of England. All patients gave informed consent, and the study was given approval by the London Brent Research Ethics Committee (reference 12/LO/1291). In total, 204 individuals were assessed: 17 cases had ARSACS, 13 were *SACS* mutation carriers and 174 cases had other types of ataxia, including 59 cases of FRDA, 53 with various SCAs and 17 with other genetically diagnosed ataxias. The 17 ARSACS cases were all drawn from the group of 26 patients described in Chapter 5. All are of UK descent except one who came from Switzerland from a family of Calabrian origin (patient 12 in Table 59). Note, the patient numbering system in this Chapter and Chapter 5 are not the same. Seventeen patients had various other genetically determined ataxias [ataxia with oculomotor apraxia type 2 (AOA2), hereditary spastic paraparesis type 7 (HSP7), episodic ataxia types 1 & 2 (EA1/2), fragile X tremor ataxia syndrome (FXTAS), ataxia with fibroblast growth factor mutation (FGF), Sjögren-Larsson syndrome (SLS) and ataxia with co-enzyme Q_{10} deficiency ($ACoQ_{10}D$)]. The patient with $ACoQ_{10}D$ had a pathogenic mutation in the aarF domain-containing kinase 3 gene (ADCK3). In 45 cases, the cause of ataxia was considered idiopathic, the patients previously having been assessed routinely in the NHNN Ataxia Centre and undergone clinically appropriate genetic, metabolic and other diagnostic tests. The demographic details and diagnoses of the study patients are given in Table 57.

6.2.2 Genetic Studies & Spastic Ataxia Custom Amplicon Panel

ARSACS cases were diagnosed by extracting genomic DNA from peripheral blood lymphocytes using standard procedures; subsequent analysis was performed by the diagnostic service in the Department of Human Genetics, Radboud University, Nijmegen Medical Centre, Nijmegen, Netherlands, using previously described methods (Vermeer et al. 2008). The 'non-ARSACS' cases were diagnosed using standard

diagnostic techniques in the Department of Neurogenetics, NHNN. The idiopathic cases were investigated using the same standard diagnostic techniques and were confirmed not to have *SACS* mutations using an Illumina TruSeq Custom Amplicon panel (Illumina, San Diego, CA USA) designed specifically for this study to detect mutations in genes causing spastic ataxia (de Bot *et al.* 2012). The genes on the panel are given in Table 58. Library preparation was undertaken using the manufacturer's protocol (part 15027983, revision C, August 2013) using MiSeq reagent kit version 3 with amplicons designed to cover *SACS* gene exons with 99% coverage. Subsequent amplification used the polymerase chain reaction (PCR) according to the manufacturer's protocol (Applied Biosystems Gene Amp PCR System 9700, Thermo Fisher Scientific, Waltham, MA USA) and massively parallel sequencing used the MiSeq sequencer (Illumina, San Diego, CA USA). Analysis was undertaken using the company's in-house software. The *SACS* mutation carriers were confirmed using the same technique. *SACS* gene variants are annotated according to reference sequence NM_014363.4.

Table 58: Genes on Illumina TruSeq Custom Amplicon spastic ataxia panel

Condition	Gene	Protein	Location
ARSACS (SPAX6)	SACS	Sacsin	13q12.12
Hereditary spastic paraplegia, type 7 (HSP7)	SPG7	Paraplegin	16q24.3
Hereditary spastic paraplegia, type 11 (HSP11)	SPG11	Spastacsin	15q21.1
Adult-onset Alexander disease	GFAP	Glial fibrillary acidic protein	17q21.31
Cerebrotendinous xanthomatosis (CTX)	CYP27A1	Cytochrome P450, subfamily XXVIIA, polypeptide 1	2q35
Fatty acid dehydrogenase deficiency (with brain iron accumulation)	FA2H	Fatty acid 2 dehydroxylase	16q23.1
Spastic ataxia with leukoencephalopathy (SPAX3)	MARS2	Methionyl tRNA synthetase 2	2q33.1
Autosomal recessive spastic ataxia, type 4 (SPAX4)	MTPAP	Mitochondrial poly(A) polymerase	10p11.23
Autosomal recessive spastic ataxia, type 5 (SPAX5)	AFG3L2	ATPase family gene 3-like 2	18p11.21
Adrenomyeloneuropathy	ABCD1	ATP-binding cassette, subfamily D, member 1	Xq28
Ataxia with co-enzyme Q_{10} deficiency ($ACoQ_{10}D$)	ADCK3	aarF domain-containing kinase 3	1q42.13

6.2.3 Optical Coherence Tomography

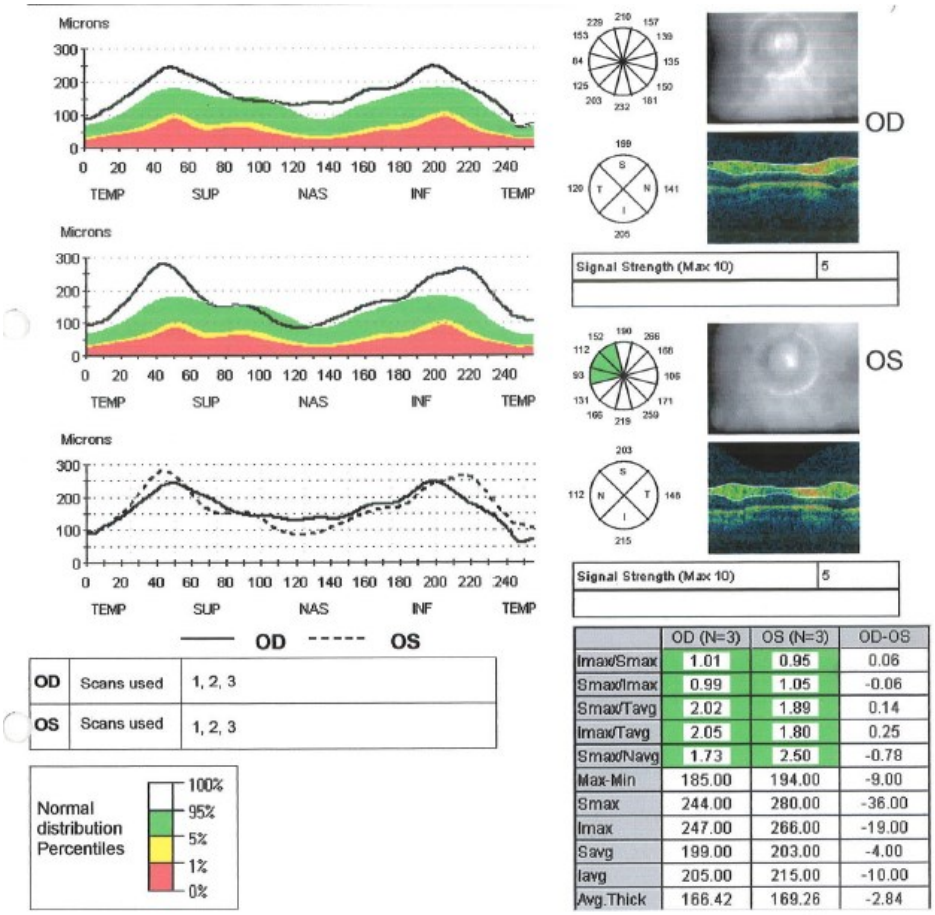
After dilating the pupils with 1% tropicamide eyedrops, the patient was seated at a time-domain OCT device (Stratus, Carl Zeiss Meditec, Dublin, CA) and asked to fixate on the device's internal target using the test eye whilst the fellow eye was occluded. Polarization was machine-optimised then the RNFL thickness was measured in a circle around the optic disc using the 'Fast RNFL Thickness' acquisition protocol (see Figure 85). OCT data were analyzed using the proprietary software (Stratus version 4.07) which provides automated estimates of RNFL thickness in each quadrant around the disc (superior, nasal, inferior and temporal) and an average measurement of RNFL thickness around the entire circumference of the optic disc. The analysis software also provides a measure of scan quality referred to as 'signal strength' on an arbitrary scale 0-10; we took the decision to accept recordings only if the signal strength was at least 5, the scan circle was well-centred on the optic disc and there was no eye movement or blink artefact. In each patient OCT scans were repeated three times per eye, and the results for both eyes averaged. In a small number of cases, imaging of both eyes was not possible for technical reasons such as inability to fixate or poor image quality.

6.2.4 Ophthalmological Examination

Patients had their best corrected visual acuity (Snellen) and colour vision (Ishihara pseudo-isochromatic plates) tested in both eyes, and then underwent a full ophthalmic examination by an experienced ophthalmologist (Dr Fion Bremner, NHNN). Fundoscopy was specifically performed to determine whether or not there was any clinically apparent abnormal thickening of the RNFL.

6.2.5 Neurological Examination

Each participant with ARSACS underwent full neurological examination as described in Chapter 5. The assessment included examination of tone, power, reflexes, sensation, coordination, eye movements and skeletal foot abnormalities. A thorough history was recorded and past medical notes and previous investigations reviewed. Age at onset was taken as the age of first symptoms compatible with the subsequent diagnosis of ARSACS as judged by the patient or in the medical notes.



	OD (N=3)	OS (N=3)	OD-OS
Imax/Smax	1.01	0.95	0.06
Smax/Imax	0.99	1.05	-0.06
Smax/Tavg	2.02	1.89	0.14
Imax/Tavg	2.05	1.80	0.25
Smax/Navg	1.73	2.50	-0.78
Max-Min	185.00	194.00	-9.00
Smax	244.00	280.00	-36.00
Imax	247.00	266.00	-19.00
Savg	199.00	203.00	-4.00
Iavg	205.00	215.00	-10.00
Avg.Thick	166.42	169.26	-2.84

Figure 85: Typical TD-OCT scan for ARSACS patient showing RNFL thickness above 95% centile in all retinal quadrants

TEMP=temporal quadrant; SUP=superior quadrant; NAS=nasal quadrant; INF=inferior quadrant
OD=right eye; OS=left eye

Table 59: Neurological, ophthalmological and OCT findings in ARSACS patients

					Neurological Features									Ophthalmological Features						Refraction		RNFL Thickness (µm)				
Patient	SACS mutation	Age at onset	Age at examination	Disease duration	Ataxia	Spasticity	Sensory loss	Weakness	Deep Tendon Reflexes	Plantar Reactions	Nystagmus	Dysarthria	Foot Abnormalities	Pupils	Ocular history	Fundal appearance	Other ophthalmic features	Snellen Acuity	Ishihara Score	RE	LE	Superior	Nasal	Inferior	Temporal	Average
1	c1144G>T; c11352_11353dup	1	30	29	+	+	(+)	(+)	N/-/++	E/E	+	+	+	N	-	RT	D	3	100	NR	NR	200	151.5	195.5	152	174.8
2	c7255_7259del; c9956_9957delAA	5	23	18	+	+	-	-	N	-	+	-	+	N	-	RT	A	3	100	NR	NR	156	120	182.5	120.5	144.9
3	c5820_5821del; c7162_7163del	1	33	32	++	++	++	+	N	E/E	+	+	+	N	-	RT	A	4	92	3	3	194.5	111.5	179	153.5	159.4
4	c8339T>G; c11675C>G; c12416T>C	48	60	12	++	+	++	+	N	E/F	+	-	-	N	-	RT	A	1	100	-0.25	0	161	152.5	190.5	84.5	147.1
5	c8339T>G; c11675C>G; c12416T>C	51	62	11	+	+	++	-	N/-	E/E	+	-	-	N	-	RT	A	3	100	NR	NR	152.5	119.5	181.5	100.5	138.5
6	c8339T>G; c11675C>G; c12416T>C	43	61	18	+	++	+	+	N/++	E/E	+	+	+	N	-	N	A	2	100	0	0	150	103	146.5	78.5	119.3
7	c8339T>G; c11675C>G; c12416T>C	35	47	12	+	+	+	-	N/++	E/E	+	-	+	N	CB	N	A	2	100	0	0	145	133	164.5	94	134.1
8	c8339T>G; c11675C>G; c12416T>C	46	60	14	+	(+)	+	+	N/-	E/E	+	-	+	N	CB	N	A	4	65	NR	NR	148	114	157	93	128.1
9	c5151dupA; c5948C>T; c6392delT	2	40	38	+	+	++	+++	-	E/E	+	-	+	N	-	N	A	4	100	-8.5	-10.25	175.5	103.5	157	120	139.4

10	c9404T>C; c11265_11266delAT	10	45	35	+	++	++	+++	-	-	+	+	+	N	LA	RT	A	4	100	2.25	2.25	188	148	225.5	121	170.7
11	c9404T>C; c11265_11266delAT	13	39	26	(+)	+	++	+	N/++	E/F	+	+	+	N	LA	N	N	4	100	NR	NR	180	147.5	195	114	158.9
12	c6078del homozygous	2	21	19	+	++	-	-	++	E/E	+	+	+	N	M	RT	K	7	100	-0.5	-1.75	190	136.5	215	143.5	171.1
13	c4226_4229del; c9404T>C	32	50	18	+	+	++	+	N/-	-	+	+	+	N	-	RT	A	NR	NR	2	2	155	126.5	180	121	145.6
14	c3149C>A; c4744G>T	5	32	27	+	(+)	-	-	N/-/++	E/-	+	++	+	N	-	RT	A	2	100	0	0	171.5	113.5	182.5	114	145.3
15	c9404T>C ; c12028C>T	3	46	43	(+)	-	+++	+	N/-	F/F	+	+	+	N	-	RT	A	4	100	0	0	195	127.5	210.5	129	165.6
16	c9404T>C ; c12028C>T	5	51	46	+	(+)	++	+	-	E/E	+	+	+	N	-	RT	A	2	100	-0.75	-0.5	177	95.5	196	108.5	144.4
17	c9956_9957del ; c10115dup	1	31	30	(+)	++	(+)	+	N/-	E/E	+	(+)	+	N	-	RT	A	4	100	0	0	196	144.5	191.5	119.5	163.0

Reference sequence NM_014363.4
Reflexes: N=normal ; - = absent ; ++ = increased
Plantar reflexes: E=extensor ; F=flexor ; - = mute
Other neurological features: - = absent ; (+) equivocally present ; + = present ; ++ strongly present ; +++ very strongly present
Foot abnormalities: PC=pes cavus ; TEV=talipes equinovarus ; TE=talipes equinus ; HT=hammer toes ; CT=claw toes
Ocular history: CB=colour blind ; LA=left eye amblyopia ; M=myopia ; - = No past ocular history
Fundal appearance : RT=retinal thickening
Other ophthalmic features : D=diplopia ; A=asymptomatic ; K=keratoconus ; N=symptomatic nystagmus
Snellen acuity: 1=6/4 ; 2=6/5 ; 3=6/6 ; 4=6/9 ; 5=6/12 ; 6=6/18 ; 7=6/24
RE=right eye; LE=left eye
NR=not recorded

6.3 Results

The 17 patients with ARSACS came from 11 families and showed 20 different pathogenic mutations (see Table 59). The patients were between the ages of 21 and 62 when assessed. Disease onset varied between 1 and 46 years, and disease duration between 11 and 46 years. In contrast to previous descriptions of the Québecois cohort of ARSACS patients (Bouchard *et al.* 1998), members of one family (patients 4-8) had disease onset in middle life (range 35-51 years). All from this family share three pathogenic mutations, none of which is found in the Québecois population (Thiffault *et al.* 2013). Two mutations (c.8339T>G and c.12416T>C) are known from studies of related carriers to cosegregate. All patients in the study had ataxia and all but one had spasticity (94.1%). Fourteen (82.4%) showed clinical evidence of sensory loss. Twelve (70.6%) had limb weakness. Deep tendon reflexes were absent in ten patients (58.8%) and hyperreflexic in six (35.3%), sometimes with mixed absent and increased reflexes in the same patient. Thirteen had extensor plantar reflexes (76.5%). Eleven patients had dysarthria (64.7%) and fifteen had skeletal foot abnormalities (88.2%). All patients displayed nystagmus.

The results of the ophthalmological assessment of patients with ARSACS are given in Table 59. In general, patients had no complaints about their eyes or vision. In a few cases there was a history of previous ophthalmic problems (two patients with amblyopia, two with congenital colour blindness and one with myopia). No patients had visual symptoms ascribable to retinal disease, with 14/17 (82.4%) patients visually asymptomatic, one patient with diplopia, one with symptomatic nystagmus and one with focussing problems caused by keratoconus. All patients except the patient with keratoconus had Snellen acuity of 6/9 or better, and only one patient was unable to identify correctly the Ishihara pseudo-isochromatic plates. In all cases the pupillary reaction to light was clinically normal. Apart from the one case with keratoconus, no other ARSACS patient was found to have any abnormality in the anterior segment of the eye on slit lamp biomicroscopy.

Fundoscopy revealed abnormal thickening of the RNFL in 13/17 patients (76.5%). None of these cases had any associated swelling or elevation of the optic nerve head, nor

were there any abnormalities seen in the overlying vitreous, retinal vessels, outer retina, retinal pigment epithelium or choroid. In four of the 17 cases of ARSACS (23.5%), fundoscopy was normal with no clinically apparent thickening of the RNFL. There was no clear correlation between genotype and retinal phenotype; indeed in patients 4-8, individuals sharing the same mutations had different fundoscopic appearances.

OCT measurements of the RNFL thickness in these ARSACS patients are shown in Table 59 (for each patient, figures shown are the mean of measurements from both eyes unless it was only possible to obtain a scan from one eye). The average RNFL thickness measurements (estimated from the entire 360^0 of the scanned circle around each disc) ranged from 119.3µm to 174.8µm, and in all cases lay above the 95% upper limit defined in the normative database provided by the manufacturer. OCT measurements of RNFL thickness were significantly lower in the four cases where fundoscopy was clinically normal (mean±SD of 130.2±8.6) than in the remaining 13 cases where it was visibly thickened (mean±SD of 156.1±12.3; p=0.001). Further analysis of OCT measurements by quadrant (superior, nasal, inferior and temporal) showed similar degrees of abnormal thickening of the RNFL in all meridians. Among the whole cohort of 17 patients with ARSACS there was a significant decline in RNFL thickness with age (linear regression coefficient R=0.624, p=0.007) and a negative correlation with age at onset (linear regression coefficient R=0.629, p=0.007) but a non-significant positive correlation with disease duration (linear regression coefficient R=0.368, p=0.146; see Figure 86).

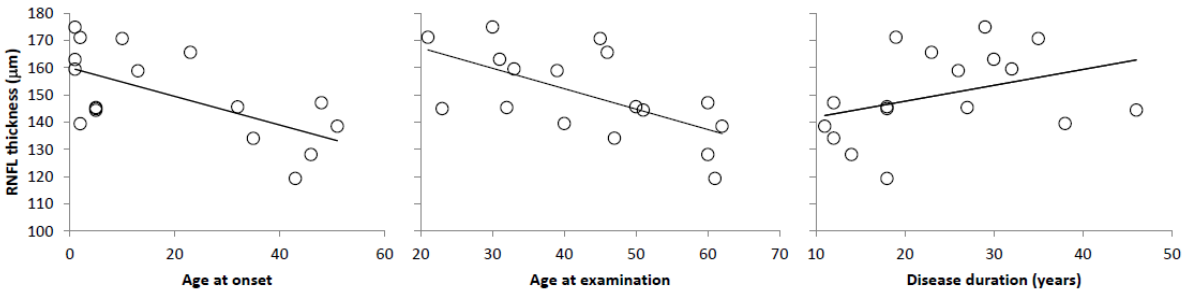


Figure 86: Correlation between average RNFL thickness for ARSACS patients and age at disease onset (left), age at examination (middle) and disease duration (right)

Thirteen asymptomatic individuals carrying heterozygous *SACS* gene mutations who were all relatives of the patients studied had RNFL thickness measured by OCT. The mean±SD average RNFL thickness was 93.8±7.5 (range 82-105.5).

One hundred and twenty-nine patients with genetically confirmed ataxias other than ARSACS were seen (FRDA n=59, SCA n=53, others n=17). Many but not all of these patients had RNFL thickness measurements below the 95% lower limit of normal compared to the machine's age-matched bank of normative data. None of these patients had average RNFL measurements ≥ 106μm whereas all the ARSACS patients had RNFL measurements ≥ 119μm, providing an absolute differentiation between cases of ARSACS and other genetically proven ataxias. However, it is important to calculate *average* peripapillary RNFL thickness, as individual *sectoral* values have greater and overlapping ranges (lowest ARSACS 78.5μm temporal quadrant; highest FRDA 150.5μm nasal quadrant).

Fundoscopy was performed in 112 patients with other types of hereditary ataxia (data not shown). As expected, a number of typical abnormalities were seen including optic atrophy (in FRDA) and pigmented maculopathy (in SCA7), but none of these patients showed thickened RNFL similar to that seen in ARSACS. Visual acuity was measured in 88 patients (FRDA n=43, SCA n=34, others n=11). There were no significant differences. Performance on Ishihara pseudo-isochromatic plates was assessed in 78 patients (FRDA n=35, SCA n=34, others n=9). There were no significant differences between groups and with ARSACS patients and carriers, although unlike ARSACS patients and carriers, there were patients in all 3 genetic groups who could not identify more than half of the plates.

Forty-five patients with were assessed, for whom no clear genetic or metabolic cause of ataxia including ARSACS had been identified (*ie* idiopathic ataxia), and none was a carrier of a *SACS* gene mutation. All patients except two had average RNFL thickness below 110μm with the lowest value of 45.5μm. Twenty-two patients also had both Snellen visual acuity and Ishihara colour plates assessed. There were no significant differences in visual acuity or colour vision between the idiopathic and any other

group, although again, unlike the patients with ARSACS and *SACS* gene mutation carriers, the idiopathic group included individuals with extremely poor vision.

Average OCT measurements of the RNFL thickness for ARSACS and all other groups studied are shown in Table 57 and displayed as box-and-whisker plots in Figure 87. The RNFL thickness of the ARSACS group was significantly different from all other groups (Mann-Whitney U test, $p<0.0005$ for all 5 tests).

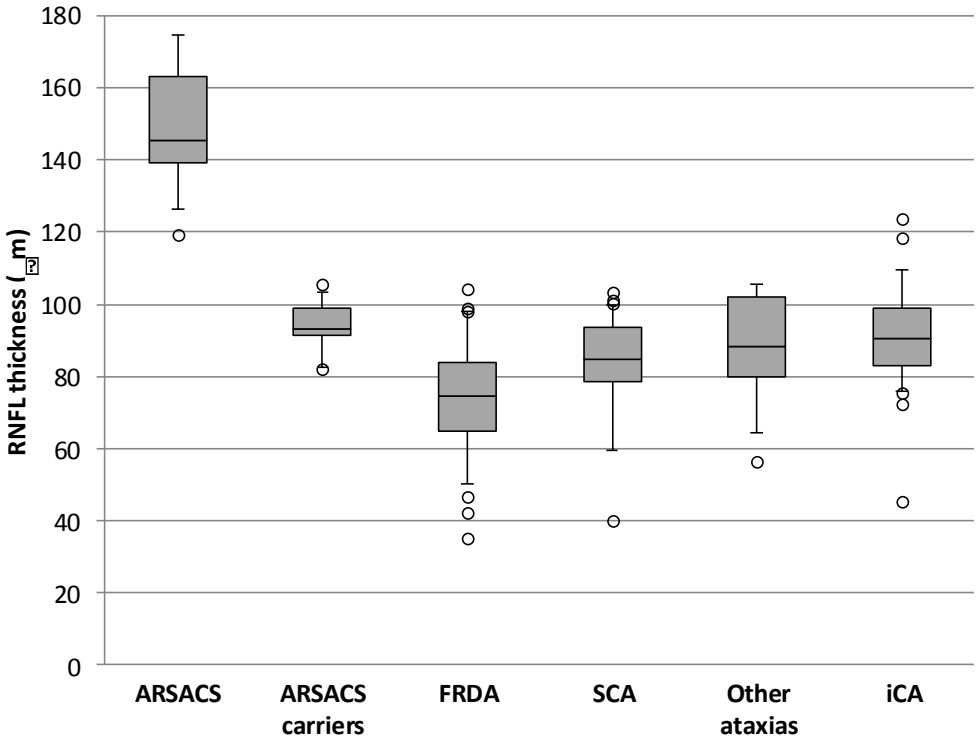


Figure 87: Box-and-whisker plots of average OCT measurements of RNFL thickness
Boxes show median and interquartile range; whiskers show 5th and 95th percentiles; points show values outside 9th and 95th percentiles.

6.4 Discussion

This is the largest cohort of patients with ARSACS and other genetic ataxias to undergo systematic evaluation by OCT and comprehensive visual assessment. The results of this study show that OCT can be used to differentiate ARSACS from other genetically diagnosed causes of progressive ataxia as well as idiopathic ataxias and can therefore be used as a reliable screening tool for deciding whether to order the genetic test in order to make a definitive genetic diagnosis. This is an important finding as, due to the large size of the *SACS* gene and number of mutations causing ARSACS, genetic testing

for ARSACS is expensive, and not available in many neurological or ophthalmological centres. An average RNFL thickness of 119μm on TD-OCT should be used as a cut-off to distinguish ARSACS from other causes of ataxia.

Table 60: Table of frequencies for RNFL thickening in ARSACS

	ARSACS genetic test positive	**ARSACS genetic test negative**	
RNFL thickness ≥119μm by TD-OCT	17 (true positive)	1 (false positive)	18
RNFL thickness <119μm by TD-OCT	0 (false negative)	173 (true negative)	173
	17	174	

Amongst patients with symptomatic ataxia, this gives a sensitivity of 100%, a specificity of 99.4% (area under ROC curve 1.000, $p<0.001$), a positive predictive value of 94.4%, a negative predictive value of 100%, a false positive rate of 0.6% and a false negative rate of 0%. Thus, RNFL thickness is an excellent test for distinguishing cases of ARSACS from non-ARSACS cases amongst a population of patients with symptomatic ataxia.

Patients should first be thoroughly assessed to exclude acute causes of RNFL thickening such as LHON and optic neuritis. Fundoscopy should always be performed to exclude papilloedema and retinitis pigmentosa as non-genetic causes of thickened RNFL. Typically, ARSACS patients also do not have accompanying visual symptoms, other than those associated with cerebellar eye signs such as nystagmus. Thus, their presence should alert the clinician to an alternative diagnosis or an additional unrelated cause of ocular pathology. Similarly, patients diagnosed with ARSACS undergoing assessment in the neurology or ophthalmology clinics can be reassured that visual loss is rare in ARSACS and unrelated to RNFL thickening.

However, caution should be exercised when assessing unaffected individuals, particularly those related to ARSACS patients, as it has previously been shown that two asymptomatic heterozygous carriers of a c.1144G>T mutation have partial thickening of the RNFL on OCT, with average values of 115.5μm. The present study found no more cases amongst a further 13 known *SACS* gene mutation carriers, suggesting that this is not a common finding in this group.

Of the patients with average RNFL thickness close to or above the proposed cut-off of 119μm, the first was a 22-year old woman with a four year history of progressive imbalance, dysarthria, dysphagia and diplopia. Imaging showed significant cerebellar atrophy and a thoracic syrinx from T9 to T11 but no brainstem or supratentorial atrophy. There was ataxia of all four limbs and brisk reflexes, but normal tone, power and sensation throughout, and she was able to walk unaided. There was no pes cavus. Eye movements showed jerky pursuits, saccadic dysmetria and gaze-evoked horizontal nystagmus. Genetic tests were negative for SCA 1,2,3,6,7,12, FRDA, AOA1, AOA2, ataxia telangiectasia, ARSACS and HSP7. There was no family history. She had average RNFL thickness of 118.6μm.

The second was a 21-year old man who was born following a prolonged delivery. He had delayed development of walking and was diagnosed with cerebellar ataxia at the age of 18 months. By the age of 10, he required a wheelchair, and he developed progressive dysarthria and dysphagia. There were some learning difficulties. He subsequently developed progressive spasticity and spasms. There was weakness and wasting of all four limbs and bilateral facial weakness. Tone was increased throughout with intermittent extensor spasms. Deep tendon reflexes were brisk in the upper limbs but absent at the ankles. There was lumbar scoliosis and pectus excavatum but no pes cavus. Eye movements showed jerky pursuits but no nystagmus or saccadic dysmetria. Pupillary reactions, visual fields, visual acuity and colour vision were normal. Neurophysiological studies showed a severe length-dependent axonal sensorimotor polyneuropathy with patchy demyelinating features. Imaging of the brain and cerebellum was normal. Genetic testing was negative for SCA 1,2,3,6,7, FRDA, BSCL2, connexion-32, HSP7, mitochondrial point mutations and ARSACS. There was no family history. Fundoscopy was visually normal. The average RNFL thickness by TD-OCT was 124.0μm. In both cases, there was no other ocular explanation for the RNFL thickening. SD-OCT confirmed global RNFL thickening but with normal foveal contour. The foveal contour is commonly obliterated in ARSACS (see Figure 83C) and so this may represent a means of distinguishing borderline cases.

It should be noted that the Illumina TruSeq Custom Amplicon technique has >99% coverage of the *SACS* gene but does not cover a short sequence of exon 10. The amplicons flank the exons by 25nt and so should detect splice-site variants, which have previously been described in ARSACS (Vermeer et al. 2008). It would not necessarily detect mutations in the 5'- and 3'-untranslated regions or within introns, and would not detect large deletions of which several have been described (Breckpot *et al.* 2008, Baets *et al.* 2010, Pyle *et al.* 2013, Terracciano *et al.* 2009, Piluso *et al.* 2011, Pilliod *et al.* 2015). The presence of two macrodeletions in the present study suggests that this may be a relatively common cause of ARSACS (and certainly more common than large deletions in FRDA). Taken together, these may therefore be alternative explanations of the two anomalous cases which could be investigated genetically. Alternative methods for detecting *SACS* gene dysfunction might include looking at downstream effects such as mRNA or sacsin protein levels.

The underlying nature of the retinal changes seen in ARSACS remains obscure. Visibly, they comprise radial white or yellow streaks emanating from the optic disc, most commonly in the interpapillomacular region, often following the retinal vessels and even obscuring them (see Figure , A & B). Although these changes were initially descriptively termed 'retinal striation' due to 'increased visibility of the retinal nerve fibers' (Bouchard et al. 1978), they subsequently took on the pseudo-pathological description of 'myelinated retinal fibers' (Bouchard 1991) or 'retinal hypermyelination' (Takiyama 2006, Gücüyener et al. 2001, Ogawa *et al.* 2004, Prodi et al. 2013). However, there is no published pathological or other evidence to support these appellations. Myelin is not normally present in the human retina. Myelination of optic nerve axons occurs by migration of oligodendrocyte progenitors along long axons, starting at the lateral geniculate body and ceasing at the *lamina cribrosa* of the optic nerve head where they are prevented from penetrating the retina probably by a dense aggregation of astrocytic processes (Hunter *et al.* 1997, Fitzgibbon & Nestorovski 1997). The intraretinal course of the retinal ganglion axons is therefore not usually myelinated.

However, myelinated axons *are* observed in the human retina in approximately 1% of cases (Straatsma *et al.* 1981), giving rise to the syndrome of (persistent) myelinated

retinal nerve fibres (SMRNF) which can also be associated with structural or developmental abnormalities of the retina, eye, brain or skull, or neoplastic disorders (see Figure 88). They usually cause a visual field defect, myopia or amblyopia (Tarabishy *et al.* 2007). As in the present series, these clinical features are not typically seen in ARSACS. When the eyes of patients with ARSACS are directly compared with those of patients with known myelinated retinal nerve fibres, a number of differences are apparent. In SMRNF, RNFL thickening is only seen where myelinated nerve fibres are visible, whereas in ARSACS RNFL thickening is more widespread, includes the macula causing filling of the foveolar depression and is present even in subjects without visible RNFL thickening. In SMRNF, the myelinated nerve fibres cause posterior shadowing on OCT obscuring the deeper layers because of hyperreflectivity which is not seen in ARSACS. These findings suggest the material deposited in the retina is different in the two cases (Vingolo et al. 2011, Desserre et al. 2011). Furthermore, nerve conduction studies are usually consistent with an axonal neuropathy with demyelinating features (Peyronnard *et al.* 1979, Prodi et al. 2013, Gazulla et al. 2012, Takiyama 2006). This was seen in the patients in the present series. Thus, depletion of myelin rather than overproduction seems to be the common pathological feature of ARSACS.

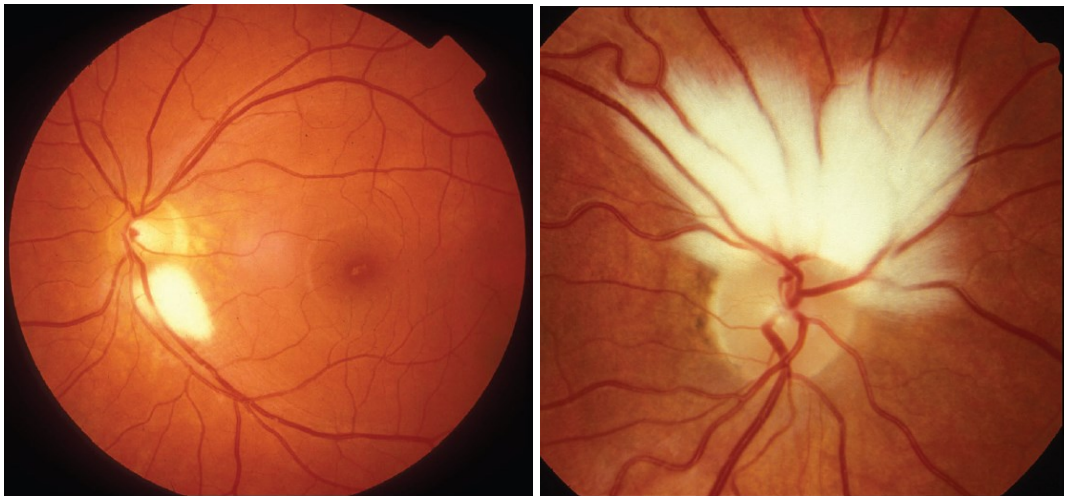


Figure 88: Myelinated retinal nerve fibres
Image from (Bruce *et al.* 2007)

Different groups have therefore proposed the alternative terms ‘pseudomyelination’ (Vingolo et al. 2011) or ‘RNFL hypertrophy’ (Garcia-Martin et al. 2013, Pablo et al. 2011) to describe the retinal appearance in ARSACS. The simpler and more descriptive

term ‘thickened retinal nerve fibre layer’ may be more appropriate (Nethisinghe et al. 2011). A greater understanding of these changes may help elucidate the underlying pathophysiological processes in this condition.

In summary, the genetic test for ARSACS is expensive, technically difficult and not widely available. In contrast, OCT is cheap, fast and widely available. This study shows that OCT is a sensitive and specific tool for identifying characteristic retinal changes in patients with ARSACS and can be used to distinguish ARSACS from other genetically diagnosed and idiopathic forms of ataxia. Its routine use in the assessment of suspected cases of ARSACS even in the absence of fundoscopic changes is advisable. The underlying pathophysiology of these changes remains obscure but there is increasing evidence that the thickness is not related to excess myelin.

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Afterword

This thesis synthesizes clinical and genetic studies in two rare and devastating autosomal recessive ataxic syndromes. 167 patients with FRDA were recruited as part of the UK wing of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) making this the largest natural history study of FRDA ever undertaken in the UK. The wider European study has now recruited more than 650 patients with the UK wing recruiting the largest cohort. The baseline clinical data from this project have recently been published (Reetz *et al.* 2015) including all the cases described in this thesis. This forms the largest clinical study of FRDA in the world literature.

The baseline clinical and demographic data for the 167 FRDA patients recruited and studied as part of the UK wing of EFACTS are described in detail in the Results section 2.3 of Chapter 2 of this thesis. Follow-up data for 125 patients after one year and 116 after two years were obtained, and data on disease progression as defined by the SARA, INAS, ADL and SDFS are described in detail in the Results section 2.3.11. The validity of these rating scales is discussed in section 2.4.5 and their ability to capture disease progression discussed in section 2.4.6. In essence, the ADL provides a reliable symptom-based assessment of disease involvement in FRDA, and the SARA, a reliable examination-based assessment. The raw INAS data provide a large amounts of useful supportive information which are statistically anaylzable but the INAS count is far less relevant in FRDA because of its dependence on non-ataxic features (which are less prominent in FRDA and therefore probably not the predominant determinants of disability). A large proportion of patients are unable to undertake all parts of the SCAFI which therefore introduces a significant ceiling effect and makes it less reliable in FRDA. In particular, more than half of the patients in the study were unable to complete the 8mTW because of wheelchair dependence. The SDFS provides a good overall marker of disability but provides little scope for further analysis as it is a simple 7-point score. The SARA, ADL and SDFS were all sufficiently sensitive to capture disease progression at 2 years but the INAS count failed to detect any statistically significant change over this period. Overall therefore, this thesis finds that the SARA is the best

rating scale for the assessment of objective examination-based disease involvement and change in FRDA, whilst the ADL provides the best assessment if subjective disease involvement.

Previous pharmacological trials in FRDA have concentrated on two pathological mechanisms, namely directly increasing frataxin expression and ameliorating the downstream effects of frataxin depletion, particularly by decreasing oxidative stress and improving mitochondrial function (Wilson 2012, Perlman 2012, Strawser *et al.* 2014). Agents aimed at increasing frataxin production have included erythropoietin, pioglitazone, resveratrol and interferon-γ. One group administered a protein formed by fusing the transduction domain of the transactivator of transcription (TAT) protein with the frataxin molecule (TAT-frataxin) producing encouraging results in cell culture and mouse models of FRDA (Vyas *et al.* 2012). More recently, strategies aimed at decreasing pathological heterochromatin formation using histone deacetylase inhibitors have been used to increase frataxin production (Sandi *et al.* 2014). In the future, further techniques may include gene therapy, stem cell therapy and RNA-based treatments.

Strategies aimed at improving mitochondrial dysfunction have included oral iron-chelating agents such as deferiprone which prevent mitochondrial iron accumulation and decrease oxidative stress (Wilson 2012, Perlman 2012, Strawser et al. 2014). A number of other anti-oxidant agents have been employed including vincerinone and oxigon, but by far the greatest number of human trials has been conducting on co-enzyme Q_{10} and its synthetic analogue, idebenone. These have been comprehensively reviewed by the author (Parkinson *et al.* 2013). Although initial results were encouraging, these have not been replicated in large, multicentre, longitudinal trials. Further techniques at ameliorating the consequences of increased oxidative stress in FRDA include the use of deuterated polyunsaturated fatty acids which helps prevent free-radical-mediated damage to membranes (Cotticelli *et al.* 2013).

This thesis provides some evidence as to which rating scales might be useful in future trials. This is based on data from the 116 patients who were seen both at baseline and the second follow-up visit. An increase (deterioration) of 1.33±3.14 (mean±SD) was

seen in the SARA over this 2 year period, representing normal disease progression. For a therapeutic agent to reduce disease progression by 50% (or 0.67 SARA points) would require a sample size of at least 240 patients, based on a power of 80% (*ie* an 80% probability of rejecting the null hypothesis) and a significance level of 5% (using Altman's nomogram with standardized difference of $2\delta/\sigma_d^2$=0.67 for the paired t-test, where δ is the smallest mean difference and δ_d the standard deviation) (Petrie & Sabin 2005, Altman 1982). Similarly, an increase (deterioration) of 2.04±3.22 was seen in the ADL over the 2 years of the study. To detect a 50% reduction in progression (or 1.02 ADL points) would require at least 75 patients. An increase (deterioration) of 0.29±0.63 was seen in the SDFS over 2 years. To detect a 50% reduction in progression would require at least 140 patients. No statistically significant change in the INAS count was detected over the 2 years of the study and so it is not possible to comment on the sample size required to detect a 50% reduction in progression according to this rating scale. These large sample sizes are required because of the large standard deviation when compared to the mean change in each case. Larger sample sizes would be required to detect more modest and perhaps more realistic reductions in disease progression, indicating, as discussed in 2.4.6 above, that these rating scales may not be appropriate to detect change in such a slowly progressive condition in which there is considerable background variation.

Seven patients in the EFACTS part of the study were compound heterozygotes bearing one pathological GAA expansion and one pathogenic point mutation. In addition, six further patients were identified from the records of the NHNN, making a total of thirteen compound heterozygotes. These cases are discussed in Chapter 3. The clinical features of these patients were studied and compared to those of the patients with two GAA expansions, showing that increased frequency of hyperreflexia, and lower frequency of square wave jerks, dysarthria, dysphagia and possibly broken pursuit eye movements and wheelchair-bound status are associated with the compound heterozygous state as compared to those with two pathological GAA expansions. Of the clinical rating scales only the SARA came close to detecting a difference between the two groups suggesting a lesser degree of ataxia.

Eleven patients had previously described *FXN* mutations (c.389G>T/p.Gly130Val [*n*=9] and c.2T>C/p.Met1Thr [*n*=2]). Two novel mutations were identified. The c.357_378dup22/p.Phe127fsX mutation introduces a premature termination codon in exon 3 of the *FXN* gene by a frameshift mechanism, and represents the first duplication mutation described in FRDA. The c.493_494CG>GA/p.Arg165Asp mutation causes a novel missense change at a known mutational hot-spot in exon 5a which *in silico* techniques predict to be pathogenic.

The clinical and genetic features of a final case (patient 'D') are discussed in which the most likely explanation of the patient's genetic abnormality is that of a compound heterozygote with a pathological GAA expansion and a large deletion. This case would constitute the twelfth case described in the world literature and the first in the UK. The discovery of this case has important implications for genetic testing as currently employed laboratory techniques may not distinguish certain compound heterozygous macrodeletions from homozygotes carrying two equally sized GAA expansions.

The prevalence of compound heterozygous exonic deletions amongst a sample of 1768 cases referred to the NHNN over a ten year period with a possible diagnosis of FRDA, was studied using the multiplex ligation-dependent probe amplification (MLPA) technique. The sample included forty cases with one pathological GAA expansion of which 11 also carried a pathogenic point mutation. Of the remaining 29 cases, nine were subsequently found to be asymptomatic leaving twenty symptomatic cases. DNA was available for 18 cases. The MLPA study was successful in 16 cases in none of which was an exonic deletion discovered. This result indicates that exonic deletions are not a common cause of FRDA, and this test should not routinely be offered for individuals with ataxia and a single pathological GAA expansion unless there is a very strong clinical suspicion of FRDA. Patient 'D' discussed above also did not show an exonic deletion using this technique which has implications for the sensitivity of the test and the composition of the probemix which is currently commercially available. As part of this study, the rate of compound heterozygotes in the sample was 5.3%, and that of carriers 1.1 to 1.6%.

Twenty-six patients with ARSACS were recruited into a natural history study of that condition. This represents the largest single centre prospective natural history case series of this very rare disease described outside Québec, and the first large systematic clinical and genetic study of ARSACS in the UK. The only larger study was recently published combining cases from twelve French centres with 47 patients from eight different countries (Pilliod *et al.* 2015). In the present study, nine novel mutations were identified which included four nonsense mutations (c.4226_4229delATGA/p.Asn1409Thr fsX, c.6078delT/p.Ala2026Ala fsX, c.10115dupC/p.Ser3372Ser fsX and c.12028C>T/p.Gln4010X) and five missense mutations (c.623G>T/p.Ser208Ile, c.3149C>A/p.Ala1050Asp, c.4723C>T/p.Arg1575Trp, c.8339T>G/p.Phe2780Cys and c.12416T>C/p.Leu4139Ser). These cases are discussed in Chapter 4 including a detailed discussion of the *in silico* predictions of pathogenicity for the missense mutations.

The parallel natural history studies of FRDA and ARSACS in which the participants underwent substantially the same clinical assessment afford the possibility of comparing these two similar conditions. Table 61 shows the comparison of clinical rating scales between the two condition, as well as ages of significant events. There was no difference in age at onset, but the ARSACS patients were significantly older when seen as part of the study and so had significantly longer disease duration. A smaller proportion of the ARSACS patients were wheelchair-bound (15.4%) compared to the FRDA patients (51.5%) and the age at becoming wheelchair-bound was significantly later for ARSACS. The FRDA patients had significantly greater total SARA sores indicating a greater burden of ataxia despite shorter disease duration. Figure 89 compares the mean vales for the individual subscores of the SARA. It can be seen that all subscores are greater in FRDA than ARSACS, but the subscores for speech, sitting and upper limb ataxia are approximately twice as great for FRDA as ARSACS. The INAS count was greater in the ARSACS than the FRDA patients. Figure 90 shows the individual components of the INAS count and it can be seen that the difference is largely driven by the greater incidence of hyperreflexia, extensor plantar reactions and spasticity in the ARSACS patients. The SDFS was slightly greater in the FRDA patients but neither the differences in INAS count nor SDFS survived Bonferroni correction.

Figure 91 shows the individual measurements of eye movement abnormalities within the INAS. This shows that nystagmus is more common in ARSACS, whilst square wave jerks, saccadic dysmetria and slowing of saccades are more common in FRDA. Broken smooth pursuits are very common in both conditions. Figure 92 shows a summary of muscle power measurements derived from the SNE as described in Chapters 2 and 5 (based on the MRC rating scale of muscle power). This shows that weakness is usually greater in FRDA than ARSACS, the lower limbs are affected more than the upper and the distal muscles, more than the proximal.

Figure 93 shows sensory loss derived from the SNE. This shows that there are comparable levels of pin prick loss between the two conditions, but that vibration sense and proprioception are much more affected in FRDA than ARSACS. In particular, there is very little upper limb vibrational and proprioceptive sensory loss in ARSACS.

Table 61: Comparison of age data and clinical rating scales for FRDA & ARSACS

	FRDA			ARSACS			Mann-Whitney	
	Mean	**SD**	***n***	**Mean**	**SD**	***n***	**Z**	***p*†**
AAO	13.7	9.6	167	15.0	17.4	26	-1.647	0.100
AAE	34.3	13.2	167	43.3	13.7	26	-3.185	0.001
DD	20.5	11.2	167	28.5	12.9	26	-3.006	0.003
AAW	21.9	8.7	86	43.4	16.8	5	-2.881	0.004
SARA	22.5	10.0	166	16.0	7.7	26	-3.280	0.001
INAS	5.0	1.6	166	5.7	1.9	26	-2.075	0.038
ADL	15.2	8.4	164	12.2	6.1	26	-1.677	0.094
SDFS	5.0	1.3	167	4.4	1.2	26	-2.801	0.050

AAO=Age at onset; AAE=age at examination; DD=disease duration; AAW=age at wheelchair-bound
SARA=Total SARA; INAS=INAS count; ADL=total ADL
SD=Standard deviation
†Bonferroni correction reduces significant p value to 0.05/8=0.00625

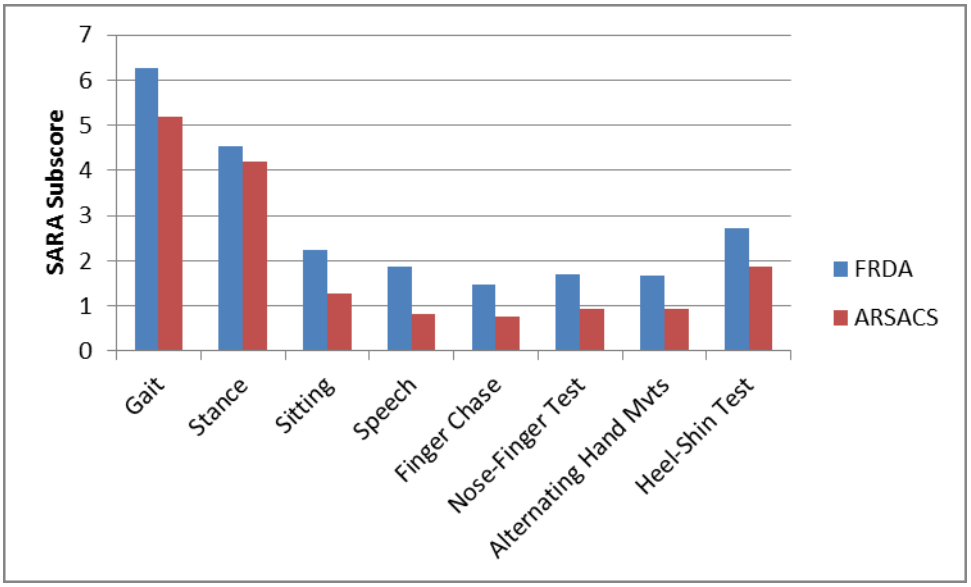

Figure 89: Comparison of SARA subscore values between FRDA & ARSACS
ARSACS patients are consistently less severely affected than FRD patients

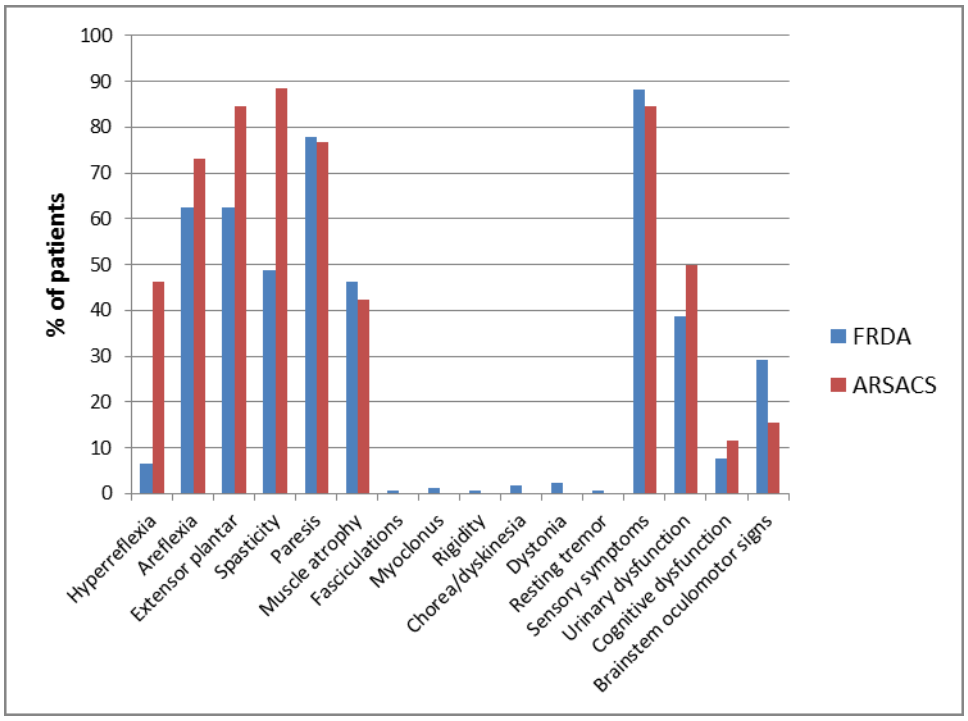

Figure 90: Comparison of components of the INAS count between FRDA & ARSACS
Pyramidal features such as hyperreflexia, spasticity and extensor plantar reflexes are more prominent in ARSACS than FRDA patients; weakness, wasting and sensory loss are similar; brainstem oculomotor signs are commoner in FRDA than ARSACS

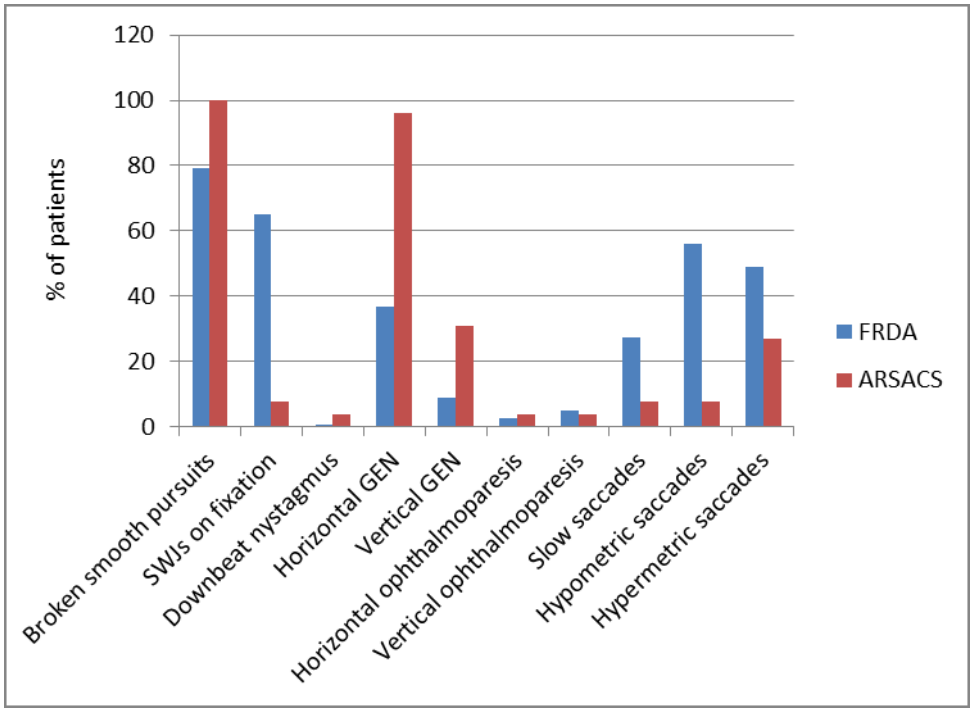

Figure 91: Comparison of eye movement abnormalities between FRDA & ARSACS
SWJs, saccadic dysmetria and slowed saccades are commoner in FRDA than ARSACS; nystagmus is commoner in ARSACS than FRDA; broken pursuits are common in both FRDA and FRDA (GEN=gaze-evoked nystagmus; SWJ=square wave jerk)

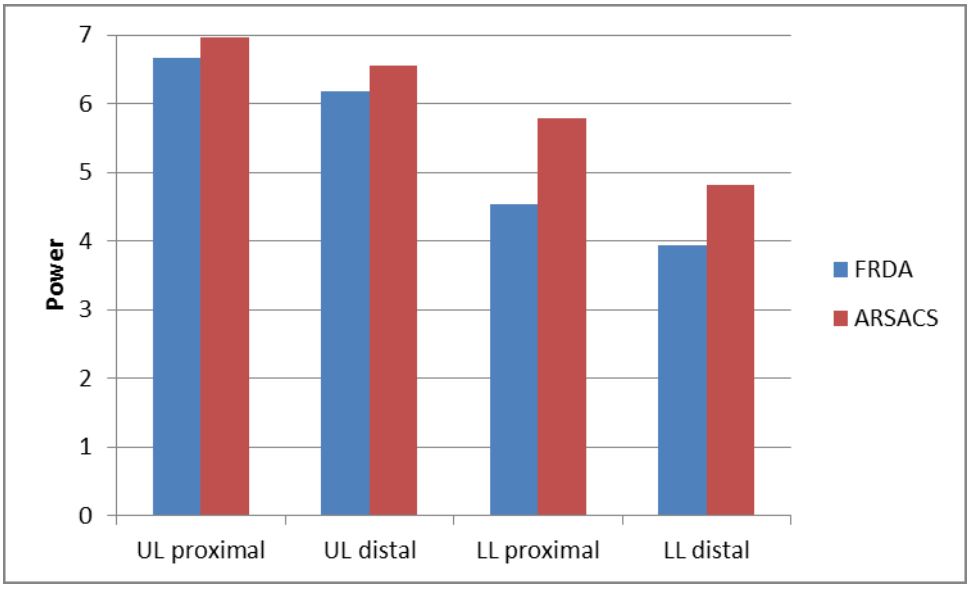

Figure 92: Comparison of muscle power between FRDA & ARSACS
FRDA patients are consistently more severely affected than ARSACS. In both condition, the LLs are more affected than ULs, and distal muscles are more affected than proximal (UL=upper limb; LL=lower limb. For explanation of scale, see Chapter 2 Method 2.2.2)

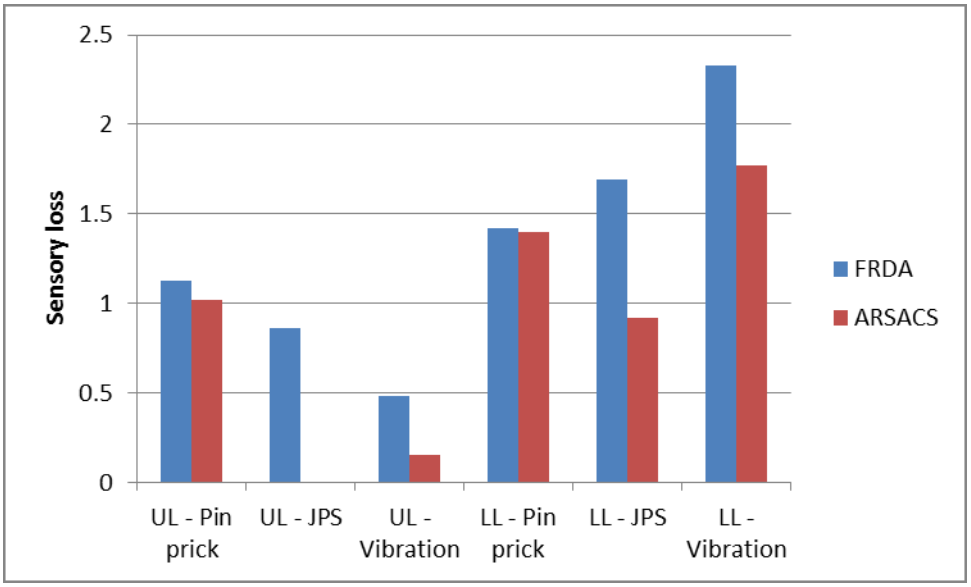

Figure 93: Comparison of sensory loss between FRDA & ARSACS
Sensory loss is consistently more pronounced in FRDA than ARSACS; in both conditions, the LLs are more affected than the ULs. (UL=upper limb; LL=lower limb. For explanation of scale, see Chapter 2 Method 2.2.2)

Thus, progression to loss of mobility is much less common in ARSACS than in FRDA. Speech, swallow and upper limb manual dexterity are also less commonly affected than in FRDA. Broken smooth pursuits and horizontal gaze-evoked nystagmus are almost universal in ARSACS, but square wave jerks are rarely observed. Broken smooth pursuits are also commonly seen in FRDA. The absence of square wave jerks and the lower frequency of slow saccades and saccadic dysmetria are clear differences from FRDA. The pattern of weakness is very similar between FRDA and ARSACS, although it is more severe in the FRDA patients. In both conditions, the lower limbs are more affected than the upper limbs, and the distal muscles more than the proximal. Sensory loss is much more prominent in FRDA. In particular, involvement of posterior column-related sensory loss is much more common in FRDA, and this tends to involve both upper and lower limbs, as opposed to being much more restricted to the lower limbs in ARSACS. The greater frequency of pyramidal features in ARSACS, notably spasticity and hyperreflexia, is another distinguishing feature which is usually only seen in late-onset atypical forms of FRDA. In addition diabetes and cardiomyopathy are not features of ARSACS.

In Chapter 6, the phenomenon of retinal nerve fibre (RNFL) thickening in ARSACS was investigated. Fundoscopic abnormalities were identified amongst the first descriptions

of ARSACS (Bouchard *et al.* 1978) but appeared to be less common in non-Québecois cases. Ocular coherence tomography (OCT) has proved to be a more sensitive tool for detecting this feature. However, it was unclear whether RNFL thickening was present in all cases of ARSACS and whether its presence was specific to ARSACS. There is a pressing need for a surrogate test for ARSACS as the genetic test is expensive, time-consuming and not readily available. The advent of massively parallel sequencing panels has reduced the cost of genetic testing somewhat (Németh *et al.* 2013), but these tools remain expensive and are still restricted to specialist units. The present study found that RNFL thickening was present in all 17 cases studied (range 119.3-174.8µm) and not present in 129 patients with other genetically diagnosed ataxic syndromes (range 35.1-106.3µm). The two groups did not overlap at all. It has previously been reported that asymptomatic heterozygous carriers of *SACS* gene mutations might also have RNFL thickening (Nethisinghe *et al.* 2011). None of the thirteen carriers in the present study had RNFL thickening (range 82.0-105.5µm). However, amongst the 45 cases with idiopathic ataxia studied (and no *SACS* gene mutations), two cases had RNFL thickness of greater than 109µm. Thus, amongst the very restricted population of patients with symptomatic ataxia under investigation in a tertiary hospital specialist ataxia clinic, this test has a sensitivity of 100% and a specificity of 99.4%.

A further feature of this study which was not discussed at length in the thesis for pressures of time and space was the development of a massively parallel sequencing panel for the diagnosis of spastic ataxias. This was primarily used in Chapter 6 to determine whether any of the idiopathic ataxia cases had *SACS* gene mutations, and also to determine the genetic status of the asymptomatic carriers. However, the original pool of patients who had undergone OCT scanning as idiopathic cases from the ataxia clinic included the four cases of HSP7 and one case of ataxia with co-enzyme Q_{10} deficiency (although she was known to have co-enzyme Q_{10} deficiency on biochemical testing). No other positive cases were found from the genes listed in the Method in Chapter 6. Thus, amongst 50 patients studied, four (8%) had HSP7 and one (2%) had ACoQ_{10}D caused by an ADCK3 mutation. Ataxia is known to be common in HSP7, in one large series affecting 57% of patients (van Gassen *et al.* 2012). The present study

confirms several very recent studies showing that HSP7 is a common cause of undiagnosed ataxia (Pfeffer *et al.* 2015, Choquet *et al.* 2015). In the UK, the p.Ala510Val mutation is the commonest cause (Roxburgh *et al.* 2012). In the current study all four patients carried the mutation c.1529C>T/p.Ala510Val as two different compound heterozygotes. Three had the mutation c.1450_1457del/p.484_486del fs, and one had the mutation c.1904C>T/p.Ser635Lys (Ref Seq NM_003199). Thus, testing for HSP7 mutations should be considered in a patient with ataxia and spasticity and no other clear genetic explanation. This perhaps could be the topic of a further research project.

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Great thanks are due to Charles Galea, Aamira Huq, Martin Delatycki and Marguerite Evans-Galea whose figures I used in Chapter 3 relating to frataxin structure and the effects of point mutations thereon. These are taken from our paper on that subject (Galea *et al.* 2015) which I certainly would not have been able to compose myself. I also feel compelled to thank two inanimate entities which have helped drag me through the painful world of statistics: I heartily recommend Medical Statistics at a Glance (Petrie A & Sabin C, Wiley-Blackwell, Chichester, UK, 2009) and the Laerd Statistics website (https://statistics.laerd.com), the latter particularly for unfathoming the mysteries of SDSS.

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Finally, I would like to express my gratitude to the patients, family members and carers who have so generously given their time to this project. We really appreciate their effort, particularly given that for many patients with these devastating conditions, it takes a singular act of endurance to travel to clinic, sometimes from far afield, and because of the way the cogs of research grind slowly on, any benefit from their participation may well accrue only to those who come after.

Various libraries have accommodated me during the monkish seclusion of writing up. At the end of this long process, the words of the printer of the first printed book in the English language seem apt. William Caxton went to Cologne in 1470 to learn the art of printing. There, he worked in a small attic translating Le Fèvre's *Recueil des histoires de Troye*. In the preface he wrote, 'my pen is worn, my hand weary and not steadfast, mine eyes dimmed with over-much looking on the white paper.' With slight modifications for technology, the process remains essentially unchanged after a further five and a half centuries!

Appendix

- Clinical Report Forms (EFACTS proforma)
- Instructions for SARA, INAS count, ADL questionnaire & SCAFI

Date seen :
Visit : BL FU1 FU2 FU3

EFACTS Pseudonimization Data

The participant's unique pseudonym is created electronically from the identification data once and never stored permanently on an electronic hard drive. The pseudonym's creation is u**nique**, **secure** and **not invertible.**

Umlauts and other special characters are automatically converted into a unique spelling.

Please **print the data** after creation of the pseudonym for **your** confidential medical documentation. That is the only way to **re-identify** the subject's personal data.

First name :	
Last name at birth :	
Date of birth (dd.mm.yyyy) :	
Hospital no :	
City/place of birth :	
Mother’s maiden name :	

Pseudonym assigned :	

Pseudonym _____-_____-_____
Baseline Follow-up

Demographics

Date data obtained: D D . M M . Y Y Y Y

Year of birth: _____

Country of birth: _____

Age: _____

Sex: ☐ male ☐ female

Children: ☐ yes ☐ no

If yes, how many: _____

Marital status:

☐ single
☐ married
☐ widowed
☐ divorced
☐ separated
☐ in a relationship

Patient ethnic group:

☐ Caucasian
(Underline subgroup: North-Western European, Eastern European, Southern European, North American European, Other regions, Mixed, Unclassified)
☐ South American
☐ African
(Underline subgroup: African African, North African, North American African, Afro-Caribbean, Other, Mixed, Unclassified)
☐ Asian
(Underline subgroup: South Asian, East Asian, Middle Eastern, Other, Mixed, Unclassified)
☐ Australian
(Underline subgroup: Australia, New Zealand, Other, Mixed, Unclassified)
☐ Native American
(Underline subgroup: Middle America, South America, Other, Mixed, Unclassified)
☐ Jewish
☐ Mixed
☐ Unclassified
☐ Other

<u>Education level and employment</u>

ISCED education level:
Please see definitions for your country online

☐ ISCED 0
☐ ISCED 1
☐ ISCED 2
☐ ISCED 3
☐ ISCED 4
☐ ISCED 5
☐ ISCED 6

Years of education: _____

Employment: ☐ yes ☐ no

If yes, is employment ☐ Full time
☐ Part time

Onset

First symptoms of FA

When: _____ age

Symptoms at onset:

Scoliosis	☐ yes	☐ no
Cardiomyopathy	☐ yes	☐ no
Instability	☐ yes	☐ no
Falls	☐ yes	☐ no
Diabetes mellitus	☐ yes	☐ no
Other	☐ yes	☐ no

If yes, please specify: _____
_____

Problems during neonatal period: ☐ yes ☐ no

If yes, please specify: _____
_____

Delayed motor milestones: ☐ yes ☐ no

If yes, please specify: _____
_____

Impaired physical abilities during infancy: ☐ yes ☐ no

If yes, please specify: _____
_____

Diagnosis

When was FA suspected by a physician: _____ age

Genetic diagnosis established? ☐ yes ☐ no

When? _____ age

Where: _____

Result: Allele 1: _____/ Allele 2: _____ GAA-repeats

Point mutation: _____
_____

EFACTS
Variable Items

Pseudonym _____-_____-_____
Baseline Follow-up

Various Items

General

Date data obtained D D . M M . Y Y Y Y

Severity Index

Disability Stage:

- ☐ No functional handicap but signs at examination
- ☐ Mild, able to run, walking unlimited
- ☐ Moderate, unable to run, limited walking without aid
- ☐ Severe, walking with one stick
- ☐ Walking with two sticks
- ☐ Unable to walk, requiring wheelchair
- ☐ Confined to bed

Gait

Permanently wheelchair bound: ☐ yes ☐ no
If yes, since when: _____ age

Permanent support for walking: ☐ yes ☐ no
If yes, since when: _____ age

Intermittent support for walking: ☐ yes ☐ no
If yes, since when: _____ age

Falls: ☐ yes ☐ no
If yes, since when: _____ age

Additional Symptoms/History

Visual impairment: ☐ yes ☐ no
Glasses since when: _____ age

Glasses effective: ☐ yes ☐ no

Glasses ineffective since: _____ age

Partial or complete hearing loss: ☐ yes ☐ no
Hearing aid since when: _____ age

Hearing aid effective: ☐ yes ☐ no

Hearing aid ineffective since: _____ age

Dyspnoea: ☐ yes ☐ no
If yes, limitation: ☐ Slight limitations of physical activity
☐ Marked limitations of physical activity
☐ Unable to carry out any physical activity

Since when: _____ age

Palpitations: ☐ yes ☐ no
Comments (start of palpitations, duration…) _____
_____

Since when: _____ age

Chest pain: ☐ yes ☐ no
Comments (describe pain: site, duration,…): _____
_____

Since when: _____ age

Additional Symptoms/History Continued

Syncope: ☐ yes ☐ no
Comments (describe syncope, frequency,…): _____
_____

Since when: _____ age

Family History

Are there any family members affected by FA? ☐ yes ☐ no
If yes, list a) *degree of relationship* (spouse, child, sibling, parent, grandparent, aunt/uncle, niece/nephew, cousin), b) *sex* (m/f), c) *blood relation* (no, identical, first degree, second degree), d) *year of birth*, e) *age at onset*, f) *age at death* (if deceased), g) *pseudonym* (if relative is part of EFACTS).

1. _____
2. _____
3. _____
4. _____
5. _____

Total no. of siblings: _____

Are there any family members affected by other types of ataxias? ☐ yes ☐ no
If yes, list a) *degree of relationship* (spouse, child, sibling, parent, grandparent, aunt/uncle, niece/nephew, cousin), b) *sex* (m/f), c) *blood relation* (no, identical, first degree, second degree), d) *year of birth*, e) *age at onset*, f) *age at death* (if deceased), g) *pseudonym* (if relative is part of EFACTS).

1. _____
2. _____
3. _____
4. _____
5. _____

Laboratory

Fasting glucose _____ (include unit)

HbA1c _____ (include unit)

Other Medical Diagnosis

Diabetes mellitus ☐ yes ☐ no ☐ unknown Since: _____ age
Type 1 ☐ Type 2 ☐

Metabolic/Endocrine ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Cardiovascular disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Hypertension ☐ yes ☐ no ☐ unknown Since: _____ age

Pulmonary Disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Gastrointestinal Disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Hepatobiliary Disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Hemato/Lymphatic ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Other Medical Diagnosis Continued

Allergy/Immunologic ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Renal Disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Gynecologic/Urologic Disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Psychiatric Disorder ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Clinical depression within the past year: ☐ yes ☐ no ☐ unknown

History of depressive disorder diagnosis: ☐ yes ☐ no ☐ unknown

Depression first diagnosed: _____ age

Neurologic (other than disease under study) ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

ENT ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Ophthalmological ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Dermatological Disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Musculoskeletal ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Autoimmune Disease ☐ yes ☐ no ☐ unknown Since: _____ age
Specify: _____

Other ☐ yes ☐ no ☐ unknown
Specify: _____ Since: _____ age
Specify: _____ Since: _____ age
Specify: _____ Since: _____ age
Specify: _____ Since: _____ age
Specify: _____ Since: _____ age

Pseudonym _____-_____-_____
Baseline Follow-up

Medication

FA related Drugs

	Drug	Since [mm.yyyy]	Ongoing	End [mm.yyyy]	Dose [w. unit]	Timing [per day/week/month]	Administration [p.o.,i.v., s.c., i.a.]
1.	_____	____.____	☐	____.____	_____	_____	_____
2.	_____	____.____	☐	____.____	_____	_____	_____
3.	_____	____.____	☐	____.____	_____	_____	_____
4.	_____	____.____	☐	____.____	_____	_____	_____
5.	_____	____.____	☐	____.____	_____	_____	_____

Please use space at the bottom of this form for additional information.

Concomitant Medication

	Drug	Since [mm.yyyy]	Ongoing	End [mm.yyyy]	Dose [w. unit]	Timing [per day/week/month]	Administration [p.o.,i.v., s.c., i.a.]
1.	_____	____.____	☐	____.____	_____	_____	_____
2.	_____	____.____	☐	____.____	_____	_____	_____
3.	_____	____.____	☐	____.____	_____	_____	_____
4.	_____	____.____	☐	____.____	_____	_____	_____
5.	_____	____.____	☐	____.____	_____	_____	_____
6.	_____	____.____	☐	____.____	_____	_____	_____
7.	_____	____.____	☐	____.____	_____	_____	_____
8.	_____	____.____	☐	____.____	_____	_____	_____
9.	_____	____.____	☐	____.____	_____	_____	_____
10.	_____	____.____	☐	____.____	_____	_____	_____

Please use space at the bottom of this form for additional information.

Vitamin Supplementation

	Vitamin	Since [mm.yyyy]	Ongoing	End [mm.yyyy]	Dose [w. unit]	Timing [per day/week/month]	Administration [p.o.,i.v., s.c., i.a.]
1.	_____	____.____	☐	____.____	_____	_____	_____
2.	_____	____.____	☐	____.____	_____	_____	_____
3.	_____	____.____	☐	____.____	_____	_____	_____
4.	_____	____.____	☐	____.____	_____	_____	_____
5.	_____	____.____	☐	____.____	_____	_____	_____

Please use space at the bottom of this form for additional information.

EFACTS
Medication

Pseudonym _____-_____-_____
Baseline Follow-up

Participation in Drug Trials

	Name of the study	FA related	Start Date [dd.mm.yyyy]	Ongoing	End Date [dd.mm.yyyy]	Start Dose [w. unit]	End Dose [w. unit]
1.	_____	☐	____.____._____	☐	____.____._____	_____	_____
2.	_____	☐	____.____._____	☐	____.____._____	_____	_____
3.	_____	☐	____.____._____	☐	____.____._____	_____	_____
4.	_____	☐	____.____._____	☐	____.____._____	_____	_____
5.	_____	☐	____.____._____	☐	____.____._____	_____	_____

Please use space at the bottom of this form for additional information.

EFACTS
Variable Items

Pseudonym _____-_____-_____
Baseline Follow-up

Drug consumption

Legal, recreational and illegal drugs: ☐ yes ☐ no

Alcohol consumption: ☐ yes ☐ no

If yes, number of alcohol units/week: ☐ none ☐ 1-20 ☐ 21-30 ☐ >30
[see conversion table]

Nicotine at present: ☐ yes ☐ no

If yes, what: ☐ Cigarettes ☐ Pipe ☐ Cigar

Number per day: _____ Since: _____ year

Nicotine in the past: ☐ yes ☐ no

If yes, what: ☐ Cigarettes ☐ Pipe ☐ Cigar

Since: _____ year To: _____ Number per day: _____

Amphetamines: ☐ yes ☐ no
[only at current consumption]
Usage: ☐ Occasional ☐ Regular

Cannabis/Marijuana: ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

Cocaine: ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

Ecstasy (MDMA): ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

Flunitrazepam (Rohyopnol ®): ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

GHB (Gamma-Hydroxybutyrate): ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

Ketamine: ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

LSD: ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

Opiates (e.g. Heroin): ☐ yes ☐ no

If yes, usage: ☐ Occasional ☐ Regular

Other: ☐ yes ☐ no

If yes, which: _____

Usage: ☐ Occasional ☐ Regular

Pseudonym _____-_____-_____
Baseline Follow-up

Cardio

Which ancillary tests have been performed?

Echocardiography

☐ yes ☐ no

When: _____ age

Septum thickness: _____ mm

Intraventricular thickness: _____ mm

Functional ejection fraction: _____ %

Electrocardiogramm

☐ yes ☐ no

When: _____ age

Sinusal rhythm: ☐ yes ☐ no

Repolarization abnormalities ☐ yes ☐ no

Q-waves: ☐ yes ☐ no

Arrhythmia: ☐ yes ☐ no

Diagnosis: _____
_____

Left ventricular hypertrophy ☐ yes ☐ no

Conduction abnormalities ☐ yes ☐ no

Diagnosis: _____
_____

Pace maker: ☐ yes ☐ no

Since when: _____ age

Present cardiologic diagnosis

Cardiac hypertrophy: ☐ yes ☐ no

Arrhythmias: ☐ yes ☐ no

Hypertension: ☐ yes ☐ no

Ischemic cardiopathy: ☐ yes ☐ no

Other cardiopathy
(valvular disease, …): ☐ yes ☐ no

Specify: _____
_____
_____

Pseudonym _____-_____-_____
Baseline Follow-up

General

Examination

Scoliosis: ☐ yes ☐ no

If yes, is scoliosis ☐ mild ☐ moderate ☐pronounced

Operation: ☐ yes ☐ no

When: _____ age

Pes cavus: Right: ☐ yes ☐ no

☐ ++ ☐ + ☐ (+)

Operation: ☐ yes ☐ no

When: _____ age

Left: ☐ yes ☐ no

☐ ++ ☐ + ☐ (+)

Operation: ☐ yes ☐ no

When: _____ age

Blood pressure: _____ /_____ mmHg
(after sitting for 5 minutes)

Pulse rate: _____ /min

Body weight: _____ kg

Body height: _____ cm

Neuropsychological testing

Phonemic verbal fluency (Letter F) _____ No. of words

Phonemic verbal fluency (LetterA) _____ No. of words

Activities of Daily Living

Speech
- ☐ normal
- ☐ mildly impaired, no communication problems
- ☐ moderately impaired, is asked to repeat from time to time
- ☐ heavily impaired, is frequently asked to repeat
- ☐ mostly incomprehensible

Swallowing
- ☐ normal
- ☐ rare choking
- ☐ frequent choking
- ☐ soft food required
- ☐ feeding tube/gastrostomy

Cutting food/ Use of Cutlery
- ☐ normal
- ☐ mildly slowed and clumsy, but no assistance required
- ☐ can cut most dishes, but slowed and clumsy, some assistance required
- ☐ dishes have to be cut by caregiver, can eat slowly
- ☐ has to be fed

Dressing
- ☐ normal
- ☐ mildly slowed and clumsy, but no assistance required
- ☐ intermittent assistance in buttoning etc. Or: Alterations of the procedures: sits down for getting dressed, does not wear ties anymore…
- ☐ considerable assistance required, can still do some things by himself/herself
- ☐ helpless

Personal hygiene
- ☐ normal
- ☐ mildly slowed and clumsy, but no assistance required
- ☐ assistance in showering and bathing required Or: considerably slowed, uses special auxiliary devices
- ☐ assistance in washing, brushing teeth, combing and using WC facilities required
- ☐ completely depending on help

Falls
- ☐ none
- ☐ infrequent falls (< once a month)
- ☐ intermittent falls (> once a month)
- ☐ falls several times per week or requires devices
- ☐ not capable to walk or stand

Walking
- ☐ normal
- ☐ mild difficulties, experiences unsteadiness
- ☐ moderate difficulties, needs little or no help
- ☐ severe gait disturbances, needs help or devices (cane, walker, Zimmer frame)
- ☐ incapable of walking even with support

Sitting
- ☐ normal
- ☐ mild truncal imbalance, backrest not required
- ☐ cannot sit without backrest
- ☐ capable of sitting only with considerable support (special chair, fixation)
- ☐ incapable of sitting

Bladder function
- ☐ normal
- ☐ mild urinary retention or mild urge incontinence (< once a month)
- ☐ moderate urinary retention or moderate urge incontinence (>= once a month) or infrequent urinary incontinence (< once a week)
- ☐ frequent urinary incontinence (>= once a week)
- ☐ complete loss of bladder function, needs intermittent catheterization or permanent catheter

INAS
(Inventory of Non-Ataxia Symptoms)

Clinical Findings

Reflexes:

	Normal	hyperreflexia	areflexia
1. Biceps (BTR)	☐	☐	☐
2. Patellar (PTR)	☐	☐	☐
3. Achilles (ATR)	☐	☐	☐

4. Extensor plantar reflex: ☐ none ☐ unilateral ☐ bilateral

Motor symptoms:

5. Spasticity	none	mild	mod	severe
Gait	☐	☐	☐	☐
Upper Limbs	☐	☐	☐	☐
Lower Limbs	☐	☐	☐	☐
6. Paresis	none	mild	mod	severe
Face/tongue	☐	☐	☐	☐
UL proximal	☐	☐	☐	☐
UL distal	☐	☐	☐	☐
LL proximal	☐	☐	☐	☐
LL distal	☐	☐	☐	☐
7. Muscle atrophy	none	mild	mod	severe
Face/tongue	☐	☐	☐	☐
UL proximal	☐	☐	☐	☐
UL distal	☐	☐	☐	☐
LL proximal	☐	☐	☐	☐
LL distal	☐	☐	☐	☐
8. Fasciculations	none	mild	mod	severe
Face/tongue	☐	☐	☐	☐
Upper limbs	☐	☐	☐	☐
Lower limbs	☐	☐	☐	☐
9. Myoclonus	none	mild	mod	severe
Face/tongue	☐	☐	☐	☐
Trunk	☐	☐	☐	☐
Upper limbs	☐	☐	☐	☐
Lower limbs	☐	☐	☐	☐
10. Rigidity	none	mild	mod	severe
Axial	☐	☐	☐	☐
Upper limbs	☐	☐	☐	☐
Lower limbs	☐	☐	☐	☐
11. Chorea/Dyskinesia	none	mild	mod	severe
Face/tongue	☐	☐	☐	☐
Neck	☐	☐	☐	☐
Trunk	☐	☐	☐	☐
Upper Limbs	☐	☐	☐	☐
Lower Limbs	☐	☐	☐	☐
12. Dystonia	none	mild	mod	severe
Face/tongue	☐	☐	☐	☐
Neck	☐	☐	☐	☐
Trunk	☐	☐	☐	☐
Upper Limbs	☐	☐	☐	☐
Lower Limbs	☐	☐	☐	☐
13. Resting tremor	none	mild	mod	severe
	☐	☐	☐	☐

Sensory symptoms:

14. Impaired vibration sense	none (8/8)	mild (>5/8)	mod (2-5/8)	severe (<2/8)
Right foot	☐	☐	☐	☐
Left foot	☐	☐	☐	☐

Ophthalmological findings:

Testing of fixation and smooth pursuit	no	yes
15. Broken up smooth pursuit	☐	☐
16. Square wave jerks on fixation	☐	☐
17. Downbeat-nystagmus on fixation	☐	☐
18. Gaze evoked-nystagmus on horizontal testing	☐	☐
19. Gaze evoked-nystagmus on vertical testing	☐	☐
20. Ophthalmoparesis on horizontal gaze	☐	☐
21. Ophthalmoparesis on vertical gaze	☐	☐
Testing of fast saccades	no	yes
22. Slowing of saccades	☐	☐
23. Hypometric saccades	☐	☐
24. Hypermetric saccades	☐	☐
Testing of visual function	no	yes
25. Impaired visual activity	☐	☐

Reported Abnormalities

26. Double vision	☐ none	☐ mild	☐ mod	☐ severe/constant
27. Dysphagia	☐ none	☐ mild	☐ mod	☐ severe/tube feeding
28. Urinary dysfunction	☐ none	☐ mild	☐ mod	☐ severe/catheter
29. Cognitive impairment	☐ none	☐ mild	☐ mod	☐ severe

30. Episodic vertigo
- ☐ none
- ☐ mild (< 1x/year)
- ☐ mod (>= 1x/year)
- ☐ severe (>= 1x/month)

31. Speech problems
- ☐ none
- ☐ mild (only when tired or after moderate amounts of alcohol)
- ☐ mod (spontaneously, but intermittent)
- ☐ severe (permanent)

32. Problems with handwriting
- ☐ none
- ☐ mild (a bit worse than used to be)
- ☐ mod (definitely worse than used to be, but legible)
- ☐ severe (legibility impaired)

33. Spontaneouscramps
- ☐ none
- ☐ mild (< 1x/month)
- ☐ mod (>= 1x/month)
- ☐ severe (>= 1x/week)

34. Other abnormal clinical findings or reported abnormalities: _____
_____
_____
_____

SARA

(Scale for the assessment and rating of ataxia)

PLEASE READ SOPs FOR SARA!

Gait:

☐ Normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed)
☐ Slight difficulties, only visible when walking 10 consecutive steps in tandem
☐ Clearly abnormal, tandem walking > 10 steps is not possible
☐ Considerable staggering, difficulties in half-turn, but without support
☐ Marked staggering, intermittent support of the wall required
☐ Severe staggering, permanent support of one cane or light support by one arm required
☐ Walking > 10m only with strong support (two special canes or stroller or accompanying person)
☐ Walking < 10m only with strong support (two special canes or stroller or accompanying person)
☐ Unable to walk, even supported

Stance:

☐ Normal, able to stand in tandem for > 10s
☐ Able to stand with feet together without sway, but not in tandem for > 10s
☐ Able to stand with feet together for > 10s, but only with sway
☐ Able to stand for > 10s without support in natural position, but not with feet together
☐ Able to stand for > 10s in natural position only with intermittent support
☐ Able to stand for > 10s in natural position only with constant support of one arm
☐ Unable to stand for > 10s even with constant support of one arm

Sitting:

☐ Normal, no difficulties sitting > 10s
☐ Slight difficulties, intermittent sway
☐ Constant sway, but able to sit > 10s without support
☐ Able to sit for > 10s only with intermittent support
☐ Unable to sit for > 10s without continuous support

Speech disturbance:

☐ Normal
☐ Suggestion of speech disturbance
☐ Impaired speech, but easy to understand
☐ Occasional words difficult to understand
☐ Many words difficult to understand
☐ Only single words understandable
☐ Speech unintelligible/anarthria

Finger chase:

Right	Left	
☐	☐	No dysmetria
☐	☐	Dysmetria, under/overshooting target < 5cm
☐	☐	Dysmetria, under/overshooting target < 15cm
☐	☐	Dysmetria, under/overshooting target > 15cm
☐	☐	Unable to perform 5 pointing movements

EFACTS
SARA

Pseudonym _____-_____-_____
Baseline Follow-up

Nose-finger test:

Right	Left	
☐	☐	No tremor
☐	☐	Tremor with an amplitude < 2cm
☐	☐	Tremor with an amplitude < 5cm
☐	☐	Tremor with an amplitude > 5cm
☐	☐	Unable to perform 5 pointing movements

Fast alternating hand movements:

Right	Left	
☐	☐	Normal, no irregularities (performs < 10s)
☐	☐	Slightly irregular (performs < 10s)
☐	☐	Clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs < 10s
☐	☐	Very irregular, single movements difficult to distinguish or relevant interruptions, performs > 10s
☐	☐	Unable to complete 10 cycles

Heel-shin slide:

Right	Left	
☐	☐	Normal
☐	☐	Slightly abnormal, contact to shin maintained
☐	☐	Clearly abnormal, goes off shin up to 3 times during 3 cycles
☐	☐	Severely abnormal, goes off shin 4 or more times during 3 cycles
☐	☐	Unable to perform the task

SCAFI
(Spinocerebellar Ataxia Functional Index)

PLEASE READ THE SOPs of the timed walking test, 9-hole peg test and PATA rate test!

Handedness

Specify dominant hand: ☐ Dominant LEFT, non-dominant right
☐ Dominant RIGHT, non-dominant left

Timed walking test: 8m (25-foot) walk
8m walking test performed? ☐ yes ☐ no

If test has been performed
Assistive device: ☐ none
☐ one cane/crutch
☐ two canes/crutches
☐ wheeled walker
☐ orthesis

Did situations arise that necessitated repetition of a trial? _____
Other factors that might have affected performance? _____

Times are only given for 2 successfully completed trials:

Trial 1: _____ seconds (round to .1 second)

Trial 2: _____ seconds (round to .1 second)

Timed dexterity test: 9-hole peg test (9-HPT)
9-hole peg test performed? ☐ yes ☐ no

If test has been performed
Did situations arise that necessitated repetition of a trial? _____
Other factors that might have affected performance? _____

Dominant hand:

Trial 1: _____ seconds (round to .1 second)

Trial 2: _____ seconds (round to .1 second)

Non-dominant hand:

Trial 1: _____ seconds (round to .1 second)

Trial 2: _____ seconds (round to .1 second)

Timed speech task: PATA rate
PATA rate test performed? ☐ yes ☐ no

If test has been performed
Did situations arise that necessitated repetition of a trial? _____
Other factors that might have affected performance? _____

Counts are only given for 2 successfully completed trials:

Trial 1: _____ times

Trial 2: _____ times

CCFS

(Composite Cerebellar Functional Severity Score)

PLEASE READ THE SOPs of the 9-hole peg test and the click test!

Age: _____ years

Has test been performed? ☐yes ☐ no

If test has been performed

Dominant hand: ☐ left ☐ right

9-hole pegboard test:
Timing Dominant hand: _____ seconds

Click test:
Timing Dominant hand: _____ seconds

Factors that might have affected performance:

_____

_____

Pseudonym _____-_____-_____
Baseline Follow-up

EQ-5D

Health

Mobility:
- ☐ I have no problems in walking about
- ☐ I have some problems in walking about
- ☐ I am confined to bed

Self-care:
- ☐ I have no problems with self-care
- ☐ I have some problems washing or dressing myself
- ☐ I am unable to wash or dress myself

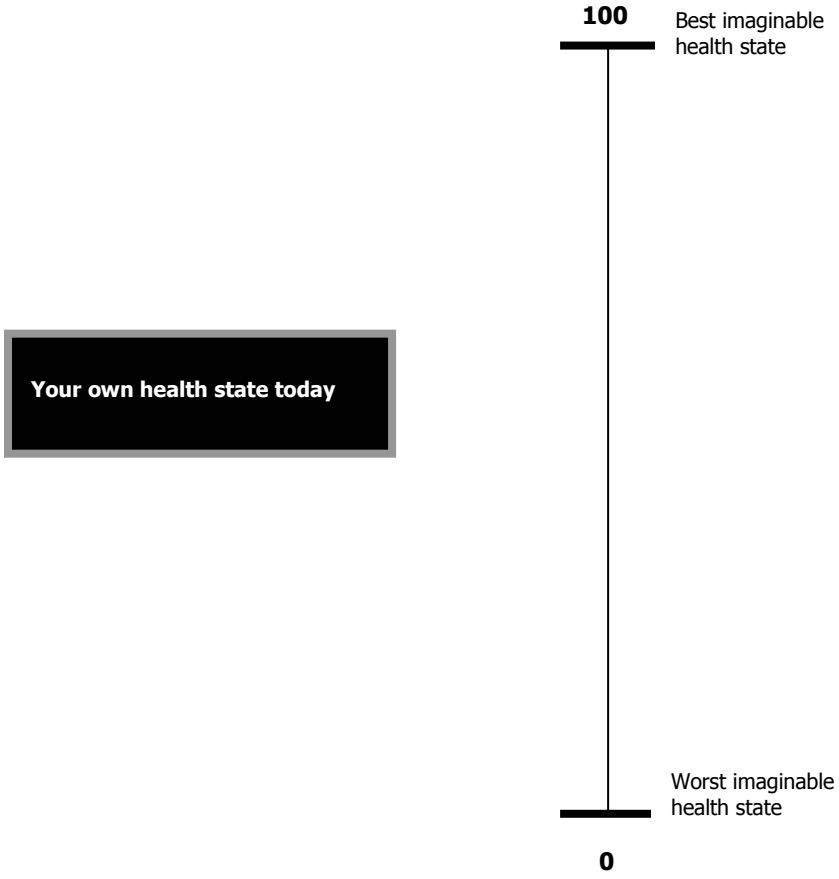
Usual activities:
(e.g. work, study, housework, family or leisure activities)
- ☐ I have no problems with performing my usual activities
- ☐ I have some problems with performing my usual activities
- ☐ I am unable to perform my usual activities

Pain/Discomfort:
- ☐ I have no pain or discomfort
- ☐ I have moderate pain or discomfort
- ☐ I have extreme pain or discomfort

Anxiety/Depression:
- ☐ I am not anxious or depressed
- ☐ I am moderately anxious or depressed
- ☐ I am extremely anxious or depressed

Health State

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.
We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.



EFACTS
EQ-5D

Pseudonym _____-_____-_____
Baseline Follow-up

Background

Have you experienced serious illness?	yes	no
in you yourself	☐	☐
in your family	☐	☐
in caring for others	☐	☐

What is your age in years? _____

Are you: ☐ male ☐ female

Are you: ☐ a current smoker ☐ an ex-smoker ☐ a never smoker

Do you now, or did you ever, work in health or social services? ☐ yes ☐ no

If yes,
in what capacity? _____

Which of the following best describes your main activity?
☐ in employment or self employment
☐ retired
☐ housework
☐ student
☐ seeking work
☐ other
If other, please specify: _____

Did your education continue after the minimum school leaving age? ☐ yes ☐ no

Do you have a Degree or equivalent professional qualification? ☐ yes ☐ no

If you know your postcode, would you please write it here: _____

Structured Neurological Examination (SNE)

Name	
Date	

Cranial Nerves		Right	Left	Comments	Grading
Pupils	Light reaction	Normal/ Abnormal	Normal/ Abnormal		0 = normal ; 1 = abnormal (specify in free text)
	RAPD	No / Yes	No / Yes		0 = no ; 1 = yes
	Snellen acuity	6 /	6 /		Give value from 6m chart
	Spectacles for Snellen	No / Yes	No / Yes		0 = no ; 1 = yes
Fields	Fields	Normal/ Abnormal	Normal/ Abnormal		Record nature of defect
Eye lids	Ptosis	No / Yes	No / Yes		0 =no ; 1 = yes
Eye mvts	Ophthalmoparesis	No / Yes	No / Yes		0 = no ; 1 = yes
	Nature of paresis	1 2 3 4 5 n/a	1 2 3 4 5 n/a		1 = CN3 ; 2 = CN4 ; 3 = CN6 ; 4 = complex ; 5 = other
	Pursuits, saccades, etc				See INAS
Trigeminal n.	Facial sensation	Normal/ Abnormal	Normal/ Abnormal		0 = normal ; 1 = abnormal (specify in free text)
	Mastication	Normal/ Abnormal	Normal/ Abnormal		0 = normal ; 1 = abnormal (specify in free text)
Facial n.	Facial palsy	Normal/ Abnormal	Normal/ Abnormal		1 = UMN ; 2 = LMN ; 3 = other (specify in free text)
Vestibulococl.	Hearing	Normal/ Abnormal	Normal/ Abnormal		0 = normal ; 1 = abnormal (specify in free text)
Palate	Palatal movement	Normal/ Abnormal	Normal/ Abnormal		0 = normal ; 1 = abnormal (specify in free text)
	Speech				As per SARA
Spinal acc. N.	SCM	/ 5	/ 5		MRC grade
	Trapezii	/ 5	/ 5		MRC grade
Tongue	Atrophy	none / mild mod / severe	none / mild mod / severe		Atrophy grade
	Tone	- - / - / NAD + / ++	- - / - / NAD + / ++		Tone grade

Other

Gait				As per SARA
Stance				As per SARA
Sitting				As per SARA
Parkinsonism		No / Yes		0 = no ; 1 = yes (specify)

Notes :

Upper Limbs

		Right	Left	Comments	Grading
Atrophy		none / mild mod / severe	none / mild mod / severe		Atrophy grade
Tone		- - / - / 0 / + / ++ R / RC / G /oth	- - / - / 0 / + / ++ R / RC / G /oth		Tone grade
Power	Shoulder abduction	/ 5	/ 5		MRC grade
	Shoulder adduction	/ 5	/ 5		MRC grade
	Elbow flexion	/ 5	/ 5		MRC grade
	Elbow extension	/ 5	/ 5		MRC grade
	Wrist extension	/ 5	/ 5		MRC grade
	Wrist flexion	/ 5	/ 5		MRC grade
	Finger extension	/ 5	/ 5		MRC grade
	Finger flexion	/ 5	/ 5		MRC grade
	1st dorsal interosseous	/ 5	/ 5		MRC grade
	Abductor digiti minini	/ 5	/ 5		MRC grade
	Abductor pollicis brevis	/ 5	/ 5		MRC grade
Reflexes	BJ	- / (+) / + / ++ / +++ / ++++	- / (+) / + / ++ / +++ / ++++		Reflex grade
	SJ	- / (+) / + / ++ / +++ / ++++	- / (+) / + / ++ / +++ / ++++		Reflex grade
	TJ	- / (+) / + / ++ / +++ / ++++	- / (+) / + / ++ / +++ / ++++		Reflex grade
	FJ	- / (+) / + / ++ / +++ / ++++	- / (+) / + / ++ / +++ / ++++		Reflex grade
	HJ	- / (+) / + / ++ / +++ / ++++	- / (+) / + / ++ / +++ / ++++		Reflex grade
Coordination	Finger-nose test				As per SARA
	Finger chase test				As per SARA
	Alternating hand mvts				As per SARA
Sensation	PP	0 1 2 3 4 5 6 7	0 1 2 3 4 5 6 7		UL Sensation grade
	JPS	0 1 2 3 4 5 6 7	0 1 2 3 4 5 6 7		UL Sensation grade
	Vibration	0 1 2 3 4 5 6 7	0 1 2 3 4 5 6 7		UL Sensation grade

Grading Scales

Atrophy : 0 = None ; 1 = Mild atrophy ; 2 = Moderate atrophy ; 3 = Severe atrophy

Tone : 1 = Highly flaccid (--) ; 2 = Flaccid (-) ; 3 = Normal ; 4 = Spastic (+) ; 5 = Highly spastic (++); 6 = Rigid (R) ;
7 = Rigid with cogwheeling (RC) ; 8 Gegenhalten (G) ; 9 = Other (specify in free text)

MRC Power : 0/5 = 0 ; 1/5 = 1 ; 2/5 = 2 ; 3/5 = 3 ; 4-/5 = 4 ; 4/5 = 5 ; 4+/5 = 6 ; 5/5 = 7

Reflexes : Absent [-] = 0 ; Present with reinforcement [(+)] = 1 ; Hyporeflexic [+] = 2 ; Normal [++] = 3 ;
Brisk [+++] -= 4 ; Brisk with clonus [++++] = 5

Plantars : 1 = down ; 2 = up ; 3 = null ; 4 = withdrawal

UL Sensation: 0 = Normal ; 1 = fingertips ; 2 = knuckles ; 3 = wrists ; 4 = elbows ; 5 = shoulders ; 6 = other (specify, eg mononeuropathy, patchy)
(Record level where sensation is first normal)

Notes :

Lower Limbs

		Right	Left	Comments	Grading
Atrophy		none / mild mod / severe	none / mild mod / severe		Atrophy grade
Tone		- - / - / 0 / + / ++ R / RC / G /oth	- - / - / 0 / + / ++ R / RC / G /oth		Tone grade
Power	Hip flexion	/ 5	/ 5		MRC grade
	Hip extension	/ 5	/ 5		MRC grade
	Hip abduction	/ 5	/ 5		MRC grade
	Hip adduction	/ 5	/ 5		MRC grade
	Knee extension	/ 5	/ 5		MRC grade
	Knee flexion	/ 5	/ 5		MRC grade
	Ankle extension	/ 5	/ 5		MRC grade
	Ankle flexion	/ 5	/ 5		MRC grade
	Ankle inversion	/ 5	/ 5		MRC grade
	Ankle eversion	/ 5	/ 5		MRC grade
	Toe extension	/ 5	/ 5		MRC grade
	Toe flexion	/ 5	/ 5		MRC grade
Reflexes	KJ	- / (+) / + / ++ / +++ / ++++	- / (+) / + / ++ / +++ / ++++		Reflex grade
	AJ	- / (+) / + / ++ / +++ / ++++	- / (+) / + / ++ / +++ / ++++		Reflex grade
	Plantars	up / down mute/withdraw	up / down mute/withdraw		Plantar grade
Coordination	Heel-shin test				As per SARA
Sensation	PP	0 1 2 3 4 5 6 7	0 1 2 3 4 5 6 7		LL Sensation grade
	JPS	0 1 2 3 4 5 6 7	0 1 2 3 4 5 6 7		LL Sensation grade
	Vibration	0 1 2 3 4 5 6 7	0 1 2 3 4 5 6 7		LL Sensation grade

Pes cavus	- (+) + ++	- (+) + ++	
Talipes equinus	- (+) + ++	- (+) + ++	
Talipes varus	- (+) + ++	- (+) + ++	
Cold feet	Yes / no		
Colour change?	white / red / blue / purple / mottled		

Grading Scales

Atrophy : 0 = None ; 1 = Mild atrophy ; 2 = Moderate atrophy ; 3 = Severe atrophy

Tone : 1 = Highly flaccid (--) ; 2 = Flaccid (-) ; 3 = Normal ; 4 = Spastic (+) ; 5 = Highly spastic (++); 6 = Rigid (R) ; 7 = Rigid with cogwheeling (RC) ; 8 Gegenhalten (G) ; 9 = Other (specify in free text)

MRC Power : 0/5 = 0 ; 1/5 = 1 ; 2/5 = 2 ; 3/5 = 3 ; 4-/5 = 4 ; 4/5 = 5 ; 4+/5 = 6 ; 5/5 = 7

Reflexes : Absent [-] = 0 ; Present with reinforcement [(+)] = 1 ; Hyporeflexic [+] = 2 ; Normal [++] = 3 ; Brisk [+++] -= 4 ; Brisk with clonus [++++] = 5

Plantars : 1 = down ; 2 = up ; 3 = mute ; 4 = withdrawal

LL Sensation: 0 = Normal ; 1 = toes ; 2 = ankles ; 3 = knees ; 4 = hips ; 5 = lower costal margin ; 6 = sternum ; 7 = other (specify) (Record level where sensation is first normal)

Rater: _____date: _____patient:_____

Scale for the assessment and rating of ataxia (SARA)

1) Gait

Proband is asked (1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and (2) to walk in tandem (heels to toes) without support.

0 Normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed)
1 Slight difficulties, only visible when walking 10 consecutive steps in tandem
2 Clearly abnormal, tandem walking >10 steps not possible
3 Considerable staggering, difficulties in half-turn, but without support
4 Marked staggering, intermittent support of the wall required
5 Severe staggering, permanent support of one stick or light support by one arm required
6 Walking > 10 m only with strong support (two special sticks or stroller or accompanying person)
7 Walking < 10 m only with strong support (two special sticks or stroller or accompanying person)
8 Unable to walk, even supported

Score _____

2) Stance

Proband is asked to stand (1) in natural position, (2) with feet together in parallel (big toes touching each other) and (3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated.

0 Normal, able to stand in tandem for > 10 s
1 Able to stand with feet together without sway, but not in tandem for > 10s
2 Able to stand with feet together for > 10 s, but only with sway
3 Able to stand for > 10 s without support in natural position, but not with feet together
4 Able to stand for >10 s in natural position only with intermittent support
5 Able to stand >10 s in natural position only with constant support of one arm
6 Unable to stand for >10 s even with constant support of one arm

Score _____

3) Sitting

Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.

0 Normal, no difficulties sitting >10 sec
1 Slight difficulties, intermittent sway
2 Constant sway, but able to sit > 10 s without support
3 Able to sit for > 10 s only with intermittent support
4 Unable to sit for >10 s without continuous support

Score _____

4) Speech disturbance

Speech is assessed during normal conversation.

0 Normal
1 Suggestion of speech disturbance
2 Impaired speech, but easy to understand
3 Occasional words difficult to understand
4 Many words difficult to understand
5 Only single words understandable
6 Speech unintelligible / anarthria

Score _____

Rater: _____date: _____patient:_____

5) Finger chase

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of proband´s reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.

- **0 No dysmetria**
- **1 Dysmetria, under/ overshooting target <5 cm**
- **2 Dysmetria, under/ overshooting target < 15 cm**
- **3 Dysmetria, under/ overshooting target > 15 cm**
- **4 Unable to perform 5 pointing movements**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

6) Nose-finger test

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.

- **0 No tremor**
- **1 Tremor with an amplitude < 2 cm**
- **2 Tremor with an amplitude < 5 cm**
- **3 Tremor with an amplitude > 5 cm**
- **4 Unable to perform 5 pointing movements**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

7) Fast alternating hand movements

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken.

- **0 Normal, no irregularities (performs <10s)**
- **1 Slightly irregular (performs <10s)**
- **2 Clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs <10s**
- **3 Very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s**
- **4 Unable to complete 10 cycles**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

8) Heel-shin slide

Rated separately for each side
Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4.

- **0 Normal**
- **1 Slightly abnormal, contact to shin maintained**
- **2 Clearly abnormal, goes off shin up to 3 times during 3 cycles**
- **3 Severely abnormal, goes off shin 4 or more times during 3 cycles**
- **4 Unable to perform the task**

Score	**R**ight	**L**eft
mean of both sides (R+L) / 2		

SOPs Scale for the assessment and rating of ataxia (SARA)

Gait:
Proband is asked

1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and
2) to walk in tandem (heels to toes) without support

Stance:
Proband is asked to stand

1) in natural position
2) with feet together in parallel (big toes touching each other) and
3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated.

Sitting:
Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.

Speech:
Speech is assessed during normal conversation.

Finger chase:
Rated separately for each side.
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50% of proband's reach. Movements have an amplitude of 30cm and a frequency of 1 movement every 2s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.

Nose finger test:
Rated separately for each side.
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90% of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.

Fast alternating hand movements:
Rated separately for each side.
Proband sits comfortably. If necessary support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precisely as possible. Movement is demonstrated by examiner at a speed of approximately 10 cycles within 7s. Exact times for movement execution have to be taken.

Heel-shin slide:

Rated separately for each side.

Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task if performed 3 times. Slide-down movements should be performed within 1s. If proband slides down without contact to shin in all three trials, rate 4.

INAS count

The INAS can be used for clinical description, but is not used as a scale and it is not appropriate to use sum scores.
However, the INAS can be transformed in a set of 16 binary variables

- rated as "present", if at least one corresponding item or location is rated as mild OR moderate OR severe.
- rated as "absent" if ALL corresponding items or locations are rated as normal
- rated as missing if at least one corresponding item or location is missing AND other corresponding items or locations rated as normal.

The 16 variables are grouped from the INAS form as follows:

1 Hyperreflexia	items 1, 2, 3
2 Areflexia	items 1, 2, 3
3 Extensor plantar	item 4
4 Spasticity	item 5
5 Paresis	item 6
6 Muscle atrophy	item 7
7 Fasciculations	item 8
8 Myoclonus	item 9
9 Rigidity	item 10
10 Chorea/dyskinesia	item 11
11 Dystonia	item 12
12 Resting tremor	item 13
13 Sensory symptoms	item 14
14 Urinary dysfunction	item 28
15 Cognitive dysfunction	item 29
16 Brainstem oculomotor signs	items 20, 21, 22

These 16 binary variables can be summed up to a simple sum score, the INAS count, that can be used as a semiquantitative variable of extracerebellar involvement in SCA.

From Jacobi H *et al* (2013) Cerebellum, **12**, 418-428

ACTIVITIES OF DAILY LIVING QUESTIONNAIRE
From FRIEDREICH'S ATAXIA RATING SCALE (FARS)
Subramony SH *et al.* (2005) Neurology, **64**, 1261-1262.

1. Speech ☐

0 - Normal
1 - Mildly affected. No difficulty being understood.
2 - Moderately affected. Sometimes asked to repeat statements.
3 - Severely affected. Frequently asked to repeat statements.
4 - Unintelligible most of the time.

2. Swallowing ☐

0 - Normal.
1 - Rare choking (< once a month).
2 - Frequent choking (< once a week, > once a month).
3 - Requires modified food or chokes multiple times a week. Or patient avoids certain foods.
4 - Requires NG tube or gastrostomy feedings.

3. Cutting Food and Handling Utensils ☐

0 - Normal.
1 - Somewhat slow and clumsy, but no help needed.
2 - Clumsy and slow, but can cut most foods with some help needed. Or needs assistance when in a hurry.
3 - Food must be cut by someone, but can still feed self slowly.
4 - Needs to be fed.

4. Dressing ☐

0 - Normal.
1 - Somewhat slow, but no help needed.
2 - Occasional assistance with buttoning, getting arms in sleeves, etc. or has to modify activity in some way (e.g. Having to sit to get dressed; use velcro for shoes, stop wearing ties, etc.).
3 - Considerable help required, but can do some things alone.
4 - Helpless.

5. Personal Hygiene ☐

0 - Normal.
1 - Somewhat slow, but no help needed.
2 - Very slow hygienic care or has need for devices such as special grab bars, tub bench, shower chair, etc.
3 - Requires personal help with washing, brushing teeth, combing hair or using toilet.
4 - Fully dependent

6. Falling (assistive device = score 3)

0 - Normal.
1 - Rare falling (< once a month).
2 - Occasional falls (once a week to once a month).
3 - Falls multiple times a week or requires device to prevent falls.
4 - Unable to stand or walk.

7. Walking (assistive device = score 3)

0 - Normal.
1 - Mild difficulty, perception of imbalance.
2 - Moderate difficulty, but requires little or no assistance.
3 - Severe disturbance of walking, requires assistance or walking aids.
4 - Cannot walk at all even with assistance (wheelchair bound).

8. Quality of Sitting Position

0 - Normal.
1 - Slight imbalance of the trunk, but needs no back support.
2 - Unable to sit without back support.
3 - Can sit only with extensive support (Geriatric chair, posy, etc.).
4 - Unable to sit.

9. Bladder Function (if using drugs for bladder, automatic score of 3)

0 - Normal.
1 - Mild urinary hesitance, urgency or retention (< once a month).
2 - Moderate hesitance, urgency, rare retention/incontinence (> once a month, but < once a week).
3 - Frequent urinary incontinence (> once a week).
4 - Loss of bladder function requiring intermittent catheterization/indwelling catheter.

TOTAL ACTIVITIES OF DAILY LIVING SCORE:

SCA Functional Index Instruction Manual

General rules of application:

- The SCAFI investigator may be a clinical investigator or technician, if training is provided.
- The SCAFI should be administered close to the beginning of the study visit to obtain optimal results, definitely before any other motor testing (e.g. SARA rating) is performed.
- The SCAFI components should be assessed in the order given below without major pauses (< 5 min) between the single trials. For discontinuation of a single component, follow the discontinue rules.
- Instructions given to the proband should follow standardized procedures as indicated below. Practice trials are limited to those stated in the EFACTS instructions.
- Efforts should be made to keep distractions during testing to a minimum (designated area for timed walk, separate room with only proband and investigator present for peg test and speech, phones turned off).
- Discourage the proband from talking throughout the 8m-walk test and 9-hole peg test.

It is important for data analysis to distinguish, if proband was unable to perform due to physical limitation or if a single component was not performed/rated due to other reason (time constraints, refusal by proband, no staff).

Any deviation from standard instruction due to proband's or examiner error or external interferences should be noted on the record form.

The stopwatch used should be counterchecked for accuracy with a different reliable stopwatch before the first SCAFI assessment.

Timed walking test: 8m walk (8MW)

Equipment:

- clearly marked 8 m line in designated unobstructed area to minimize external interference.

- stopwatch
- assistive device for walking, if needed by proband

Instruction:

The proband is directed to one end of the 8 m line and asked to walk the 8 m distance to the other end as quickly as possible but safely (Trial 1). Examiner walks along with the proband. Exact time is taken, recorded and stopwatch reset. The task is immediately administered again by having the proband walk back the same distance (Trial 2). Exact time is taken (excluding time for turning). The test is performed from standing start with both feet behind the start line (assistive device may be ahead of startline), but without stopping at the finish line. Timing begins when lead foot passes the starting line and stops when lead foot passes the finish line. Walk time is reported to within 0.1 second, rounded as needed. Maximal rest period between both trials is 5 minutes.

Probands may use assistive devices, usually their customary device. For probands with significant gait impairment, the investigator should have the proband use a wheeled walker, even if this is not the customary device (decision on assistive device to be made by neurologist). In general, non-wheeled walkers should not be used. Assistance of another person or using the wall as support is not allowed. If such attempts are made more than twice, repeat the trial or reevaluate proband for use of assistive device. The same device should be used at follow-up if possible.

Discontinue rules:

1. if proband cannot complete a trial in 3 minutes.
2. if proband cannot complete trial 2 of the timed walk after max. 5 -min rest period after trial 1, discontinue 8m-walk.

Timsed dexterity test: 9-hole peg test (9HPT)

Equipment:

- stopwatch
- solid table (not rolling bedside table)
- Rolyan 9-hole peg test apparatus(plastic one-piece model)
- (exactly) nine pegs in the peg container of the 9-hole peg board

- extra pegs to replace fallen pegs in examiner's hand
- adhesive material to anchor the apparatus on the table, e.g. Dycem™ obtained by suppliers of occupational therapy materials.

Instruction:

The pegboard is placed and secured on the table directly in front of the proband with the mould (peg container) in front of the hand that is going to be tested (i.e. to the right side, if right hand is tested). The dominant hand is tested first for two consecutive trials, immediately followed by two consecutive trials of the non-dominant hand. Handedness here refers to the hand that is used or has been used for writing the majority of time.

The following instruction is given to the proband:

"On this test, I want you to pick up the pegs one at a time, using one hand only, and put them into the holes as quickly as you can in any order until all the holes are filled. Then, without pausing, remove the pegs one at a time and return them to the container as quickly as you can. We'll have you do this two times with each hand. We'll start with your (dominant) hand. You can hold the peg board steady with your (non-dominant) hand. If a peg falls onto the table, please retrieve it and continue with the task. If a peg falls on the floor, keep working and I will retrieve it for you. See how fast you can put all of the pegs in and take them out again."

Timing begins when proband touches the first peg and stops when the last peg is removed and hits the container. Time is reported to within 0.1 second, rounded as needed. After trial 1 of the dominant hand is completed, time is recorded and stopwatch reset. Then proband is asked to perform again with the same hand.

If subject stops after having put all the pegs into the holes, examiner may prompt the subject (without interruption of timing) to continue directly with removing them one by one. If more than one is removed at a time, remind the proband to remove them one by one. Other communication throughout the test should be avoided and if proband starts talking she/he should be reminded not to talk.

If pegs drop onto the table within proband's arm reach, proband is to retrieve it. If it falls on the floor or onto the table beyond proband's reach, examiner is to retrieve it and puts it back in the container.

After trial 2 of the dominant hand, the pegboard is rotated 180° with the peg container towards the other hand and proband instructed as follows *"Now I'd like you to switch and use your (non-dominant) hand. This time you may use your (dominant) hand to stabilize the peg board."*

Two consecutive trials are performed with the non-dominant hand.

No major pause (>5 min) between all four trials.

Discontinue rule:

1. if proband cannot complete one trial in 5 minutes (i.e. 300 seconds) with dominant hand, move on to the trials with non-dominant hand.
2. if proband cannot complete one trial in 5 minutes (i.e. 300 seconds) with non-dominant hand, discontinue 9-hole-peg test.

Timed speech task: PATA rate

Equipment:

- stopwatch
- tape recorder that can play at fast and slow speed. Recordings should be done at normal (2,4 cm/sec) speed, while counting is done by playing at slow speed.

  OR
- standard PC equipped with microphone, using audio software to visualize vocalization (e.g. free download of www.audacity.sourceforge.net). In this case, time count is included in the software. **OR**
- standard PC and text software

Instruction:

The proband is asked to repeat "PATA" as quickly and distinctly as possible for 10 seconds until told to stop. Say "go" and as soon as proband starts speaking, start timer and begin counting the number of PATA repeats. After 10 seconds, stop timer and stop counting.

The test is performed two times without major pause (< 5min) inbetween.

The count of PATA repeats usually needs a technical device and can be done by different means:

1. record the test on a tape recorder and use playback at slower speed for counting the numbers of PATA between the “go” and “stop” signal.
2. record the test on PC and count the numbers of PATA repeats within 10 seconds. Slow playback and time count is inherent in the software.
3. press any key on the PC keyboard for each PATA repetition in any text software looking at the stopwatch. After 10 seconds, count the number of keystrings.
4. paper and pencil: put a mark on paper for each PATA repetition. After 10 seconds, count the number of marks.

Discontinue rule:

1. If PATA articulation is too difficult to distinguish for counting
2. If proband cannot complete 10 seconds for two consecutive trials

SOPs for Spinocerebellar Ataxia Functional Index (SCAFI)

SCAFI (Schmitz-Hübsch, Neurology 2008)

Components: 8 metre foot walk (8MW) + 9 hole peg board (9HPT) + PATA rate

8 metre foot walk (8MW) is defined at the time needed to walk 8m with any device but without help of another person or wall ´as quickly as possible but safely´. The time is measured from standing start with feet behind the start line (whereas assistive device is allowed ahead of start line). The 8MW test has to be repeated resulting in two values. Stop criterion is 180 s. If the test is not performed, please indicate the reason as ´patient unable to perform the test due to physical limitation (unabke to perform) or ´other´(other reason)

For the 9 hole peg board (9HPT), the patient is asked to complete a 9-hole peg board and to remove all pegs for each hand separately (dominant (D) and nondominant (ND) hand) with the writing hand as the dominant hand. Stop criterion is 300 s. The 9HPT test has to be repeated resulting in two values for each hand. If the test is not performed, please indicate the reason as ´patient unable to perform the test due to physical limitation (unable to perform) or ´other´(other reason).

IMPORTANT: the 9HPT design differs between SCAFI and CCFS.

For assessing the PATA rate, the proband is asked to repeat the syllable ´PATA´ within 10 seconds. The PATA rate test has to be repeated resulting in two values. If the test is not performed, please indicate the reason as ´patient unable to perform the test due to physical limitation (unable to perform)´ or ´other´(other reason).

INTRODUCTION

Verbal fluency requires the production of words within a time limit and under a specific constraint, e.g., words beginning with the letter "F" (Letter Fluency). This is an executive function task where strategies such as clustering can be implemented in order to facilitate word production. Our Letter Fluency test includes two two-minute trials using the letters F and A.

LETTER FLUENCY ADMINISTRATION

(1) Begin by reading the following instructions to the participant.

I am going to say a letter of the alphabet, and I want you to say as quickly as you can, all the words you can think of which begin with that letter. You may say any words at all, except proper names such as the names of people or places, so you could not say 'Robert' or' Rhode Island.' Also, do not use the same word again, with a different ending, such as 'eat' and 'eating.' For example, if I say 'B,' you could say 'boy,' 'book,' 'blue,' and so on. Can you think of another word beginning with the letter 'B'?"

Wait until the participant gives a word. If he succeeds, indicate that he is performing correctly and start with testing. - If the participant gives an inappropriate word, correct him, repeat the instructions, and ask him to try again.

(2) Before administering the test, read the following instructions to the participant.

"That's fine, now I am going to give you another letter and again you say all the words beginning with that letter that you can think of. Remember, no names of people or places, just ordinary words. Also, if you draw a blank, I want you to keep trying until the time limit is up. You will have two minutes for each letter."

Get the stopwatch ready. Legibly record all responses. The participant is encouraged to go as fast as he can. If there are long pauses, or if he says "that's it," it's OK to encourage the participant with comments like, ***"Any other words that you can think of?," or, "You have some time left; see if you can think of a few more words."***

In general, you should never interrupt in the middle of a trial except to give encouragement. You may interrupt the participant if it is obvious that he has misunderstood the instructions (for example, if he begins the task by using the wrong letter). Also, if the participant responds
with two proper names, remind him that proper names are not allowed after the second proper name is given.

If the participant gives a word that you may not have heard correctly or that you are not familiar with, spell the word as best you can with what you heard and mark the word with a question mark next to it. After the trial is over, you may ask the participant to repeat the word or to spell it for you. If you are unsure if a response is a real word, assume it is until the word can be looked up in a dictionary.

It is possible that the participant may give responses at a rate too fast to legibly record all responses. If this occurs, write enough of the word (e.g., the first three to five letters) that will allow you to know what that word is when you go back to it later. Finish recording any unfinished words when you have time either during pauses within the trial or after the trial.

Any word that the participant at least starts to say before the time is up is to be recorded as a response. If you say, "Stop," while the participant is saying a word, the participant may finish the word after the stopping point.

When the participant is ready to begin, say,
"Go as fast as you can. The first letter is 'F.' Ready? Go."
Start the stopwatch immediately after you say "Go" for each letter.
Allow 2 minutes seconds for each letter. After 2 minutes, say, ***"Stop."***

At the start of the second trial, say, ***"The next letter is 'A.' Go."***
After 2 minutes, say, ***"Stop."***